PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 15 March 2000 (15.03.00)	
International application No. PCT/EP99/05297	**Applicant's or agent's file reference** C 2098 PCT
International filing date (day/month/year) 23 July 1999 (23.07.99)	**Priority date** (day/month/year) 24 July 1998 (24.07.98)
Applicant SPIESS, Joachim et al	

1. **The designated Office is hereby notified of its election made:**

[X] in the demand filed with the International Preliminary Examining Authority on:

14 February 2000 (14.02.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. **The election** [X] **was**

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Nestor Santesso
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

3164395

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference (if desired) (12 characters maximum) C 2098 PCT

Box No. I TITLE OF INVENTION

Antagonists specific for the corticotropin-releasing factor receptor type 2 (CRFR2)

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

Max-Planck-Gesellschaft zur Förderung der Wissenschaften e. V.
Berlin
DE

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State (that is, country) of nationality:
DE

State (that is, country) of residence:
DE

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

SPIESS, Joachim Dr. Dr.
Max-Planck-Institut für Experimentelle Medizin
Hermann-Rein-Strasse 3
37075 Göttingen
DE

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:
DE

State (that is, country) of residence:
DE

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

Vossius & Partner
Postfach 86 07 67
81634 München
DE

Telephone No.
089-4 13 04-0

Facsimile No.
089-4 13 04-111

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Sheet No. 2

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

RUHMANN, Andreas Dr.
ANSTO
Australian Nuclear Science & Technology
Organisation, Radiopharmaceuticals Division
Private Mail Bag 1
MENAI NSW 2234, AU

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: DE

State *(that is, country)* of residence: AU

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [X] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

☐ AP **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☐ EA **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☐ OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☐	AL Albania	☐	LS Lesotho
☐	AM Armenia	☐	LT Lithuania
☐	AT Austria	☐	LU Luxembourg
☒	AU Australia	☐	LV Latvia
☐	AZ Azerbaijan	☐	MD Republic of Moldova
☐	BA Bosnia and Herzegovina	☐	MG Madagascar
☐	BB Barbados	☐	MK The former Yugoslav Republic of Macedonia
☐	BG Bulgaria		
☐	BR Brazil	☐	MN Mongolia
☐	BY Belarus	☐	MW Malawi
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☐	CH and LI Switzerland and Liechtenstein	☐	NO Norway
☐	CN China	☐	NZ New Zealand
☐	CU Cuba	☐	PL Poland
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☐	DE Germany	☐	RO Romania
☐	DK Denmark	☐	RU Russian Federation
☐	EE Estonia	☐	SD Sudan
☐	ES Spain	☐	SE Sweden
☐	FI Finland	☐	SG Singapore
☐	GB United Kingdom	☐	SI Slovenia
☐	GD Grenada	☐	SK Slovakia
☐	GE Georgia	☐	SL Sierra Leone
☐	GH Ghana	☐	TJ Tajikistan
☐	GM Gambia	☐	TM Turkmenistan
☐	HR Croatia	☐	TR Turkey
☐	HU Hungary	☐	TT Trinidad and Tobago
☐	ID Indonesia	☐	UA Ukraine
☐	IL Israel	☐	UG Uganda
☐	IN India	☒	US United States of America
☐	IS Iceland		
☒	JP Japan	☐	UZ Uzbekistan
☐	KE Kenya	☐	VN Viet Nam
☐	KG Kyrgyzstan	☐	YU Yugoslavia
☐	KP Democratic People's Republic of Korea	☐	ZW Zimbabwe
☐	KR Republic of Korea	Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:	
☐	KZ Kazakhstan		
☐	LC Saint Lucia	☐	.....
☐	LK Sri Lanka	☐	.....
☐	LR Liberia	☐	.....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) (24.07.98) 24. Juli 1998	98 11 3896.9		EP	
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): 1

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA /EPA

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	:	4
description (excluding sequence listing part)	:	32
claims	:	4
abstract	:	1
drawings	:	4
sequence listing part of description	:	
Total number of sheets	:	45

This international application is accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify)*:

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

München, 23. Juli 1999

Dr. Renate Barth
European Patent Attorney

Ba/TG/es

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
C 2098 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/05297	23/07/1999	24/07/1998
International Patent Classification (IPC) or national classification and IPC		
C07K14/435		
Applicant		
MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSC		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14/02/2000	28.11.2000
Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax +49 89 2399 - 4465	Mennessier, T Telephone No. +49 89 2399 8687

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/05297

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:

Description, pages:

1-31 as originally filed

Claims, No.:

1-33 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 25-33.

because:

☒ the said international application, or the said claims Nos. 30, 31 (partly),32,33 (each claim with respect to industrial applicability) relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*)

☒ the claims, or said claims Nos. 25, 26, 27-33 (these latter claims partly) are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: No:	Claims Claims	1-24 and 27-33 (see point 3.a) of the suppl. sheet)
Inventive step (IS)	Yes: No:	Claims Claims	1-24 and 27-33 (see point 3.a) of the suppl. sheet)
Industrial applicability (IA)	Yes: No:	Claims Claims	1-24 and 31 (see point 3,c)i)) of the suppl. sheet)

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

1. Documents cited in this report

Reference is made to the following documents which all cited in the international search report:

D1: *Cell and Tissue Research*, 283(1), 1996, 117-23
D2: *Peptides*, 6(suppl. 3), 1985, 209-13
D3: *Curr. Pharm. Des.*, 1(3), 1995, 305-16
D4: *Proceedings of the National Academy of Sciences of the United States of America*, 95(26), 1998 December 22, 15264-9

Document D4 to which the inventors contributed appears to be the non-patent literature counterpart of the present application under examination.

2. Comments with respect to item III

a) Claims 30, 31 (insofar as it relates to "*the use of claim 30*"), 32 and 33 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

b) Claims 25, 26 and 27-33, insofar as these latter claims relate to an antibody

(i) Antibodies to the antagonists as recited in claim 25 are only merely mentioned as being an aspect of the invention on page 9 of the description. No particular antibody is identified elsewhere in the description, which would recognize specifically an antagonist of the invention while being not or only poorly capable of binding to the native sauvagine from which the said antagonist is derived, and which would be distinguished from the prior art anti-sauvagine antibodies (see document D1, page 118, right-hand column; and document D2, pages 211 and 212).

(ii) Nor is any particular anti-idotypic antibody as recited in claim 26

described in the application. It has to be stressed that examples dealing with a particular antagonist are clearly inefficient at supporting claims directed to an anti-idotypic antibody directed against an antibody which is itself directed against the said antagonist. As no such anti-idotypic antibody is described, it would be simply impossible to assess whether claims relating thereto involve an inventive step.

(iii) Therefore, the subject-matter of claims 25-26 is so inadequately supported by the description that no meaningful opinion could be formed, this conclusion extending de facto to the subject-matter of claims 27-33, insofar as it is defined with reference to any of the said antibodies.

3. Comments with respect to item V

a) Preliminary remarks

The following comments with respect to novelty, inventive step and industrial applicability are made only with regard to claim 1-24 (as a whole) and claims 27-33 (only partly, i.e., only with respect to their subject-matter which does not relate to any antibody).

b) Novelty and inventive step (Article 33(2) and 33(3) PCT)

The various aspects of the invention as defined in the afore-mentioned claims relies on the design, synthesis and characterisation by the inventors of a high-affinity mCRFR2β-specific peptide antagonist. The said antagonist has its amino acid sequence based on native sauvagine. It is a truncated analog thereof lacking the 10 N-terminal amino acids. Such an analog or related truncated analogs appear not to be disclosed in the state of the art.

The technical problem solved by the invention may be regarded as the development of CRFR-2 specific antagonists to (i) permit discrimination between receptor type-specific functions, (ii) serve as a useful tool to detect CRFR2 and elucidate its functional role in the brain and peripheral organs,

and (iii) be efficient at treating a CRF-R2-associated disease.

Focusing on the development of non-peptide antagonists (see D3, page 313, right-hand column) the state of the art would lead the person skilled in the art away from the solution proposed by the invention.

Therefore, it may be acknowledged that the various aspects of the invention as defined in the afore-mentioned claims are new and involve an inventive step.

c) Industrial applicability (Article 33(4) PCT)

(i) The subject-matter of claims 1-24 and that of claim 31, insofar as it relates to "*The antagonist of any one of claims 1 to 18, or 24*", is considered to be susceptible of industrial applicability.

(ii) For the assessment of the present claims 30, 31 (insofar as it relates to "*the use of claim 30*"), 32 and 33 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be deoendent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

d) P-document

Should the various aspects of the claimed invention appear not to be entitled to the claimed priority date, document D4 which is cited in the international search report as a P-document should be taken into consideration when examining whether the invention is new and involves an inventive step.

4. Comments with respect to item VII

The Applicants are advised that some national/regional patent offices such as the EPO would object to a claim which as present claim 31 is directed to more than one object, the claimed objects being of different categories.

5. Comments with respect to item VIII

a) As it has not been explicitly specified in claim 1 that the amino acid sequence of the antagonist is based on sauvagine the claimed subject-matter is considered not to be unambiguously defined. Claim 1 is therefore objected to under Article 6 PCT.

b) In view of the comments made at point 3 above, claims 25 to 33 are objected to under Article 6 PCT.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [7]:
C07K 14/435, 16/18, A61K 38/16, 39/395, C12N 15/12, 15/63, G01N 33/53, 33/68 // C07K 105:00

A3

(11) International Publication Number: **WO 00/05253**

(43) International Publication Date: 3 February 2000 (03.02.00)

(21) International Application Number: PCT/EP99/05297

(22) International Filing Date: 23 July 1999 (23.07.99)

(30) Priority Data:
98113896.9 24 July 1998 (24.07.98) EP

(71) Applicant *(for all designated States except US)*: MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V. [DE/DE]; Berlin (DE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SPIESS, Joachim [DE/DE]; Max-Planck-Institut für Experimentelle Medizin, Hermann-Rein-Strasse 3, D-37075 Göttingen (DE). RÜHMANN, Andreas [DE/AU]; ANSTO, Australian Nuclear Science & Technology Organisation, Radiopharmaceuticals Division, Private Mail Bag 1, Menai, NSW 2234 (AU).

(74) Agent: VOSSIUS & PARTNER; Postfach 86 07 67, D-81634 München (DE).

(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(88) Date of publication of the international search report:
15 June 2000 (15.06.00)

(54) Title: ANTAGONISTS SPECIFIC FOR THE CORTICOTROPIN-RELEASING FACTOR RECEPTOR TYPE 2 (CRFR2)

(57) Abstract

The present invention relates to an antagonist of the ligand of the Corticotropin-Releasing Factor Receptor, type 2 (CRFR2) lacking the 8 to 10 N-terminal amino acids of native sauvagine. The present invention also relates to an antagonist of the ligand of the Corticotropin-Releasing Factor Receptor, type 2 (CRFR2) lacking the 11 N-terminal amino acids of native sauvagine, wherein the N-terminal amino acid of said antagonist is a charged amino acid. Furthermore, the present invention relates to a polynucleotide encoding the antagonist of the present invention, a vector comprising the polynucleotide of the present invention, and a host comprising the polynucleotide or the vector of the present invention. Also described are a method for producing the antagonist of the present invention, antibodies directed the antagonist of the present invention, as well as anti-idiotypic antibodies directed against the antibody of the present invention. The present invention also relates to pharmaceutical and diagnostic compositions comprising the antagonist, the polynucleotide, the vector, the antibody, and/or the anti-idiotypic antibody of the present invention. Furthermore, the present invention relates to a kit comprising one or more of the above mentioned compounds of the present invention and to the use of one or more of these compounds for the preparation of a pharmaceutical composition for preventing and/or treating a Corticotropin-Releasing Factor Receptor, type 2 (CRFR2)-associated disease.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

national Application No
PCT/EP 99/05297

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/435 C07K16/18 A61K38/16 A61K39/395 C12N15/12
C12N15/63 G01N33/53 G01N33/68 //C07K105:00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GONZALEZ G C ET AL: "Presence of sauvagine -like epitopes in the interrenal gland of the bullfrog Rana catesbeiana." CELL AND TISSUE RESEARCH, (1996 JAN) 283 (1) 117-23. , XP000881891 page 118, right-hand column	25-33
X	GAUDINO G ET AL: "Active peptides from amphibian skin are also amphibian neuropeptides." PEPTIDES, (1985) 6 SUPPL 3 209-13. , XP000881933 page 211, right-hand column -page 212, left-hand column	25-33

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 March 2000	12/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax (+31–70) 340–3016	Mennessier, T

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LOVENBERG, TIMOTHY W. ET AL: "Corticotropin - releasing factor receptors: inhibitors, subtypes, pharmacology, localization, and their role in central nervous system function" CURR. PHARM. DES. (1995), VOLUME DATE 1995, 1(3), 305-16 , XP000882143 page 313, right-hand column -page 314, left-hand column	1-33
A	SPIESS, JOACHIM ET AL: "Molecular properties of the CRF receptor" TRENDS ENDOCRINOL. METAB. (MAY-JUNE 1998), 9(4), 140-145 , XP000881929 the whole document	1-33
A	BEHAN, DP ET AL: "Neurobiology of corticotropin releasing factor (CRF) receptors and CRF-binding protein: implications for the treatment of CNS disorders" MOL. PSYCHIATRY (1996), 1(4), 265-277 , XP000881969 page 265 -page 268, left-hand column page 272, right-hand column -page 273	1-33
A	US 4 474 765 A (PERSEO GIUSEPPE ET AL) 2 October 1984 (1984-10-02) the whole document	1-33
P,X	RUHMANN A ET AL: "Structural requirements for peptidic antagonists of the corticotropin - releasing factor receptor (CRFR): development of CRFR2beta-selective antisauvagine-30." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 DEC 22) 95 (26) 15264-9. , XP000882801 the whole document	1-33

INTERNATIONAL SEARCH REPORT

Information on patent family members

national Application No
PCT/EP 99/05297

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4474765 A	02-10-1984	BE 896400 A	01-08-1983
		DE 3312399 A	13-10-1983
		FR 2524878 A	14-10-1983
		GB 2118190 A,B	26-10-1983
		JP 58183657 A	26-10-1983
		NL 8301281 A	01-11-1983

INTERNATIONAL SEARCH REPORT

national Application No
PCT/EP 99/05297

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/435 C07K16/18 A61K38/16 A61K39/395 C12N15/12
C12N15/63 G01N33/53 G01N33/68 //C07K105:00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
17 March 2000	12/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Mennessier, T

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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A	BEHAN, DP ET AL: "Neurobiology of corticotropin releasing factor (CRF) receptors and CRF-binding protein: implications for the treatment of CNS disorders" MOL. PSYCHIATRY (1996), 1(4), 265-277 , XP000881969 page 265 -page 268, left-hand column page 272, right-hand column -page 273	1-33
A	US 4 474 765 A (PERSEO GIUSEPPE ET AL) 2 October 1984 (1984-10-02) the whole document	1-33
P,X	RUHMANN A ET AL: "Structural requirements for peptidic antagonists of the corticotropin - releasing factor receptor (CRFR): development of CRFR2beta-selective antisauvagine-30." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 DEC 22) 95 (26) 15264-9. , XP000882801 the whole document	1-33

INTERNATIONAL SEARCH REPORT

Information on patent family members

national Application No
PCT/EP 99/05297

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4474765 A	02-10-1984	BE 896400 A	01-08-1983
		DE 3312399 A	13-10-1983
		FR 2524878 A	14-10-1983
		GB 2118190 A,B	26-10-1983
		JP 58183657 A	26-10-1983
		NL 8301281 A	01-11-1983

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
C 2098 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/05297	23/07/1999	24/07/1998
International Patent Classification (IPC) or national classification and IPC C07K14/435		
Applicant MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSC		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14/02/2000	23.11.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Mennessier, T Telephone No. +49 89 2399 8687

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/05297

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-31 as originally filed

Claims, No.:

1-33 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 25-33.

because:

☒ the said international application, or the said claims Nos. 30, 31 (partly),32,33 (each claim with respect to industrial applicability) relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☒ the claims, or said claims Nos. 25, 26, 27-33 (these latter claims partly) are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-24 and 27-33 (see point 3.a) of the suppl. sheet)
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-24 and 27-33 (see point 3.a) of the suppl. sheet)
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-24 and 31 (see point 3,c)i)) of the suppl. sheet)
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

1. Documents cited in this report

Reference is made to the following documents which all cited in the international search report:

D1: *Cell and Tissue Research*, 283(1), 1996, 117-23
D2: *Peptides*, 6(suppl. 3), 1985, 209-13
D3: *Curr. Pharm. Des.*, 1(3), 1995, 305-16
D4: *Proceedings of the National Academy of Sciences of the United States of America*, 95(26), 1998 December 22), 15264-9

Document D4 to which the inventors contributed appears to be the non-patent literature counterpart of the present application under examination.

2. Comments with respect to item III

a) Claims 30, 31 (insofar as it relates to "*the use of claim 30*"), 32 and 33 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

b) Claims 25, 26 and 27-33, insofar as these latter claims relate to an antibody

(i) Antibodies to the antagonists as recited in claim 25 are only merely mentioned as being an aspect of the invention on page 9 of the description. No particular antibody is identified elsewhere in the description, which would recognize specifically an antagonist of the invention while being not or only poorly capable of binding to the native sauvagine from which the said antagonist is derived, and which would be distinguished from the prior art anti-sauvagine antibodies (see document D1, page 118, right-hand column; and document D2, pages 211 and 212).

(ii) Nor is any particular anti-idotypic antibody as recited in claim 26

described in the application. It has to be stressed that examples dealing with a particular antagonist are clearly inefficient at supporting claims directed to an anti-idotypic antibody directed against an antibody which is itself directed against the said antagonist. As no such anti-idotypic antibody is described, it would be simply impossible to assess whether claims relating thereto involve an inventive step.

(iii) Therefore, the subject-matter of claims 25-26 is so inadequately supported by the description that no meaningful opinion could be formed, this conclusion extending de facto to the subject-matter of claims 27-33, insofar as it is defined with reference to any of the said antibodies.

3. Comments with respect to item V

a) Preliminary remarks

The following comments with respect to novelty, inventive step and industrial applicability are made only with regard to claim 1-24 (as a whole) and claims 27-33 (only partly, i.e., only with respect to their subject-matter which does not relate to any antibody).

b) Novelty and inventive step (Article 33(2) and 33(3) PCT)

The various aspects of the invention as defined in the afore-mentioned claims relies on the design, synthesis and characterisation by the inventors of a high-affinity mCRFR2ß-specific peptide antagonist. The said antagonist has its amino acid sequence based on native sauvagine. It is a truncated analog thereof lacking the 10 N-terminal amino acids. Such an analog or related truncated analogs appear not to be disclosed in the state of the art.

The technical problem solved by the invention may be regarded as the development of CRFR-2 specific antagonists to (i) permit discrimination between receptor type-specific functions, (ii) serve as a useful tool to detect CRFR2 and elucidate its functional role in the brain and peripheral organs,

and (iii) be efficient at treating a CRFR2-associated disease.

Focusing on the development of non-peptide antagonists (see D3, page 313, right-hand column) the state of the art would lead the person skilled in the art away from the solution proposed by the invention.

Therefore, it may be acknowledged that the various aspects of the invention as defined in the afore-mentioned claims are new and involve an inventive step.

c) Industrial applicability (Article 33(4) PCT)

(i) The subject-matter of claims 1-24 and that of claim 31, insofar as it relates to "*The antagonist of any one of claims 1 to 18, or 24*", is considered to be susceptible of industrial applicability.

(ii) For the assessment of the present claims 30, 31 (insofar as it relates to "*the use of claim 30*"), 32 and 33 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

d) P-document

Should the various aspects of the claimed invention appear not to be entilted to the claimed priority date, document D4 which is cited in the international search report as a P-document should be taken into consideration when examining whether the invention is new and involves an inventive step.

4. Comments with respect to item VII

The Applicants are advised that some national/regional patent offices such as the EPO would object to a claim which as present claim 31 is directed to more than one object, the claimed objects being of different categories.

5. Comments with respect to item VIII

a) As it has not been explicitly specified in claim 1 that the amino acid sequence of the antagonist is based on sauvagine the claimed subject-matter is considered not to be unambiguously defined. Claim 1 is therefore objected to under Article 6 PCT.

b) In view of the comments made at point 3 above, claims 25 to 33 are objected to under Article 6 PCT.

VOSSIUS & PARTNER
PATENTANWÄLTE
SIEBERTSTR. 4
81675 MÜNCHEN

23. Juli 1999

New PCT Patent Application
Max-Planck-Gesellschaft zur
Förderung der Wissenschaften e.V.
Our Ref.: C 2098 PCT

Antagonists specific for the corticotropin-releasing factor receptor type 2 (CRFR2)

The present invention relates to an antagonist of the ligand of the Corticotropin-Releasing Factor Receptor, type 2 (CRFR2) lacking the 8 to 10 N-terminal amino acids of native sauvagine. The present invention also relates to an antagonist of the ligand of the Corticotropin-Releasing Factor Receptor, type 2 (CRFR2) lacking the 11 N-terminal amino acids of native sauvagine, wherein the N-terminal amino acid of said antagonist is a charged amino acid. Furthermore, the present invention relates to a polynucleotide encoding the antagonist of the present invention, a vector comprising the polynucleotide of the present invention, and a host comprising the polynucleotide or vector of the present invention. Also described are a method for producing the antagonist of the present invention, antibodies directed against the antagonist of the present invention, as well as anti-idiotypic antibodies directed against the antibody of the present invention. The present invention also relates to pharmaceutical and diagnostic compositions comprising the antagonist, the polynucleotide, the vector, the antibody, and/or the anti-idiotypic antibody of the present invention. Furthermore, the present invention relates to a kit comprising one or more of the above mentioned compounds of the present invention and to the use of one or more of these compounds for the preparation of a pharmaceutical composition for preventing and/or treating a Corticotropin-Releasing Factor Receptor, type 2 (CRFR2)-associated disease.

Corticotropin-releasing factor (CRF), believed to synchronize the endocrine, autonomic, immunologic and behavioral responses to stress, was characterized as a 41-residue polypeptide (Spiess, J., J. Rivier, C. Rivier, and W. Vale, *Proc. Natl. Acad. Sci. USA* **78**:6517-6521, 1981) on the basis of its ability to stimulate the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary (Vale, W., J. Spiess, C. Rivier, and J. Rivier, *Science* **213**:1394-1397, 1981).

CRF exhibits its activity through G protein-coupled receptors. CRF receptor, type 1 (CRFR1), mainly found in pituitary and brain was cloned from human, mouse, rat, chicken, and frog (Vita, N., P. Laurent, S. Lefort, P. Chalon, J.-M. Lelias, M. Kaghad, G. Le Fur, D. Caput, and P. Ferrara, *FEBS Lett.* **335**:1-5, 1993; Chen, R., K. A. Lewis, M. H. Perrin, and W. Vale, *Proc. Natl. Acad. Sci. USA* **90**:8967-8971, 1993; Perrin, M. H., C. J. Donaldson, R. Chen, K. A. Lewis, and W. Vale, *Endocrinology* **133**:3058-3061, 1993; Chang, C.-P., R. V. Pearse II, S. O'Connell, and M. G. Rosenfeld, *Neuron* **11**:1187-1195, 1993; Yu, J., L. Y. Xie, and A. B. Abou-Samra, *Endocrinology* **137**:192-197, 1996; Dautzenberg, F. M., K. Dietrich, M. R. Palchaudhuri, and J. Spiess, *J. Neurochem.* **69**:1640-1649, 1997). cDNAs coding for two splice variants of CRF receptor, type 2, CRFR2α and CRFR2β, were cloned from brain, heart, and skeletal muscle (Lovenberg, T. W., C. W. Liaw, D. E. Grigoriadis, W. Clevenger, D. T. Chalmers, E. B. De Souza, and T. Oltersdorf, *Proc. Natl. Acad. Sci. USA* **92**:836-840, 1995; Perrin, M., C. Donaldson, R. Chen, A. Blount, T. Berggren, L. Bilezikjian, P. Sawchenko, and W. Vale. *Proc. Natl. Acad. Sci. USA* **92**:2969-2973, 1995; Kishimoto, T., R. V. Pearse II, C. R. Lin, and M. G. Rosenfeld, *Proc. Natl. Acad. Sci. USA* **92**:1108-1112, 1995; Stenzel, P., R. Kesterson, W. Yeung, R. D. Cone, M. B. Rittenberg, and M. P. Stenzel-Poore, *Mol. Endocrinol.* **9**:637-645, 1995). In rodents, CRFR2α has been exclusively found in the central nervous system (CNS), whereas CRFR2β is predominantly distributed in the periphery. In humans, both receptor subtypes have been found in the CNS (Valdenaire, O., T. Giller, V. Breu, J. Gottowik, and G. Kilpatrick, *Biochim. Biophys. Acta* **1352**: 129-132, 1997). Recently, it has been proposed that urocortin (Ucn), a natural CRF analog, is the endogenous ligand to CRFR2 (Vaughan, J., C. Donaldson, J. Bittencourt, M. H. Perrin, K. Lewis, S. Sutton, R. Chan, A. V. Turnbull, D. Lovejoy, C. Rivier, J. Rivier, P. E. Sawchenko, and W. Vale, *Nature (London)* **378**:287-292, 1995).

CRF is assumed to play a major role in a number of neuropsychiatric diseases including affective disorders, anxiety disorders, anorexia nervosa and Alzheimer's disease (Behan, D. P., S. C. Heinrichs, J. C. Troncoso, X. J. Liu, C. H. Kawas, N. Ling, and E. B. De Souza, *Nature (London)* **378**:284-287, 1995). There is substantial interest in the design and synthesis of CRF antagonists acting selectively at one of the different CRF forms. After the discovery of potent peptide antagonists based on the N-terminally truncated amino acid sequence of human/rat CRF (h/rCRF) (Rivier, J., C.

Rivier, R. Galyean A. Miranda, C. Miller, A. G. Craig, G. Yamamoto, M. Brown, and W. Vale, *J. Med. Chem.* **36**:2851-2859, 1993; Hernandez, J. F., W. Kornreich, C. Rivier, A. Miranda, G. Yamamoto, J. Andrews, Y. Tache, W. Vale, and J. Rivier, *J. Med. Chem.* **36**:2860-2867, 1993; Miranda, A., S. C. Koerber, J. Gulyas, S. L. Lahrichi, A. G. Craig, A. Corrigan, A. Hagler, C. Rivier, and J. Rivier, *J. Med. Chem.* **37**:1450-1459, 1994; Gulyas, J., C. Rivier, M. Perrin, S. C. Koerber, S. Sutton, A. Corrigan, S. L. Lahrichi, A. G. Craig, W. Vale, and J. Rivier, *Proc. Natl. Acad. Sci. USA* **92**:10575-10579, 1995; Miranda, A., S. L. Lahrichi, J. Gulyas, S. C. Koerber, A. G. Craig, A. Corrigan, C. Rivier, W. Vale, and J. Rivier, *J. Med. Chem.* **40**:3651-3658, 1997), several CRFR1-selective nonpeptidic antagonists have been developed (Chen, C., R. Dagnino, Jr., E. B. De Souza, D. E. Grigoriadis, C. Q. Huang, Kjung-Il Kim, Z. Liu, T. Moran, T. R. Webb, J. P. Whitten, Y. F. Xie, and J. R. McCarthy, *J. Med. Chem.***39**: 4358-4360, 1996; Schulz, D. W., R. S. Mansbach, J. Sprouse, J. P. Braselton, J. Collins, M. Corman, A. Dunaiskis, S. Faraci, A. W. Schmidt, T. Seeger, P. Seymour, F. D. Tingley III, E. N. Winston, Y. L. Chen, and J. Heym, *Proc. Natl. Acad. Sci. USA* **93**:10477-10482, 1996; Christos, T. E., and A. Arvanitis, *Expert Opinion On Therapeutic Patents* **8**: 143-152, 1998) which attenuate CRF-mediated seizure (Baram, T. Z., D. T. Chalmers, C. Chen, Y. Koutsoukos, and E. B. De Souza, *Brain Research* **770**: 89-95, 1997) or interleukin-1β-induced fever or exhibit anxiolytic activity *in vivo* (Lundkvist, J., Z. Chai, R. Teheranian, H. Hasanvan, T. Bartfai, F. Jenck, U. Widmer, and J.-L. Moreau, *Eur. J. Pharmacol.* **309**: 195-200, 1996). However, the fact that CRF antagonist α-helical $CRF_{(9-41)}$ exhibits different inhibitory potencies in three different *in vivo* bioassay systems (Fisher, L., C. Rivier, J. Rivier, and M. Brown, *Endocrinology* **129**:1312-1316, 1991) suggests that distinct physiological functions of endogenous CRF or Ucn are mediated via CRFR1, CRFR2 or both receptor types.

Thus, the technical problem underlying the present invention was to develop CRFR2-specific antagonists to permit discrimination between receptor type-specific functions, e.g., in the brain and peripheral organs.

The solution to said technical problem is provided by the embodiments characterized in the claims.

Accordingly, the present invention relates to an antagonist of the ligand of the Corticotropin-Releasing Factor Receptor, type 2 (CRFR2) lacking the 8 to 10 N-terminal amino acids of native sauvagine.

In accordance with the present invention the term "ligand" encompasses any molecule capable of specifically binding to the Corticotropin-Releasing Factor Receptor, type 2 (CRFR2), including, e.g., (the) naturally occurring, endogenous ligand(s) of CRFR2, or any compound(s) recombinantly or chemically synthesized or biochemically modified and capable of binding and activating CRFR2.
The term "CRFR2-specific", "CRFR2-selective", "CRFR2-specificity" or "CRFR2-selectivity" as used in accordance with the present invention denotes a value which is higher than 30, preferably higher than 45, and more preferred higher than 70, and calculated according to the equation mentioned in the legend to table 1. Thus, as used in accordance with the present invention, e.g., a "CRFR2-specific" antagonist is not meant to exclusively bind to CRFR2, but to bind to CRFR2 with an at least 30-fold, preferably 45-fold, and more preferred 70-fold higher selectivity than astressin, which exhibits a similar affinity for CRFR1 and CRFR2, in particular CRFR2β.

Studies which have been carried out in accordance with the present invention surprisingly revealed that deletion of the 8 to 10 N-terminal amino acids of native sauvagine, which is a potent non-selective activator of CRFR, renders this compound a highly specific antagonist for CRFR2.

In a preferred embodiment the antagonist of the present invention lacks the 10 N-terminal amino acids of native sauvagine.

In a more preferred embodiment the antagonist of the present invention comprises the amino acid sequence Xaa_1-Xaa_2-Leu-Leu-Arg-Lys-Met-Ile-Glu-Ile-Glu-Lys-Gln-Glu-Lys-Glu-Lys-Gln-Gln-Ala-Ala-Asn-Asn-Arg-Leu-Leu-Leu-Asp-Thr-Ile-NH_2,
wherein Xaa_1 is a neutral amino acid, and Xaa_2 is a charged amino acid.

In another more preferred embodiment of the antagonist of the present invention, Xaa_1 is a hydrophobic amino acid, and Xaa_2 is Glu or His.

In an even more preferred embodiment of the antagonist of the present invention Xaa_1 is Leu.

In a further more preferred embodiment, Xaa_1 is a polar amino acid and Xaa_2 is Glu or His.

In a still more preferred embodiment of the antagonist of the present invention Xaa_1 is Tyr.

It is envisaged that antagonists of the present invention comprising Tyr as the N-terminal amino acid can be advantageously used to be radioactively labelled with, e.g., ^{125}I. Such compounds may then be employed in in vivo or in vitro experiments for the detection of CRFR2 binding sites. Although antagonists ^{125}I-labelled via His are also encompassed by the present invention, antagonists labelled via Tyr are preferred because the labelling reaction is easier to perform from the technical point of view, and the labelled compound is more stable and, therefore, easier to handle.

In a further even more preferred embodiment of the antagonist of the present invention Xaa_1 is in the D-configuration.

In a further still more preferred embodiment of the antagonist of the present invention Xaa_1 is D-Leu or D-Tyr.

In a most preferred embodiment of the antagonist of the present invention Xaa_2 is Glu.

In another still more preferred embodiment of the antagonist of the present invention Xaa_1 is D-Phe.

In another most preferred embodiment of the antagonist of the present invention Xaa_2 is His.

In another embodiment, the present invention relates to an antagonist of the ligand of

the Corticotropin-Releasing Factor Receptor type 2 (CRFR2) lacking the 11 N-terminal amino acids of native sauvagine, wherein the N-terminal amino acid of said antagonist is a charged amino acid.

In a preferred embodiment, said charged amino acid is positively charged.

In a more preferred embodiment, said charged amino acid is His.

In another preferred embodiment, the antagonist of the present invention comprises a phenyldiazirine group coupled to the N-terminal amino acid of said antagonist.
Antagonists of the present invention comprising a phenyldiazirine group are envisaged to be used, e.g., in photoaffinity labelling experiments. Accordingly, these compounds can be advantageously used to characterize antagonistic binding sites of CRFR2β receptor systems.

In a more preferred embodiment, said phenyldiazirine group is a 4-(1-azi-2,2,2-trifluoroethyl)benzoyl (ATB)-group.

In a further embodiment the present invention relates to a polynucleotide encoding the antagonist of the present invention.
The polynucleotide of the present invention may be, e.g., DNA, cDNA, RNA or synthetically produced DNA or RNA or a recombinantly produced chimeric nucleic acid molecule comprising any of those polynucleotides either alone or in combination.

In another embodiment the present invention relates to a vector comprising the polynucleotide of the present invention.
The vector of the present invention may be, e.g., a plasmid, cosmid, virus, bacteriophage or another vector used conventionally in genetic engineering, and may comprise further genes such as marker genes which allow for the selection of said

vector in a suitable host cell and under suitable conditions.

In a preferred embodiment of the vector of the present invention the polynucleotide is operatively linked to an expression control sequence.

Said expression control sequence allows expression in prokaryotic or eukaryotic cells. Expression of said polynucleotide comprises transcription of the polynucleotide into a translatable mRNA. Regulatory elements ensuring expression in eukaryotic cells, preferably mammalian cells, are well known to those skilled in the art. They usually comprise regulatory sequences ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers, and/or naturally-associated or heterologous promoter regions.

Possible regulatory elements permitting expression in prokaryotic host cells comprise, e.g., the PL, lac, trp or tac promoter in E. coli, and examples for regulatory elements permitting expression in eukaryotic host cells are the AOX1 or GAL1 promoter in yeast or the CMV-, SV40- , RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells. Beside elements which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. Furthermore, depending on the expression system used leader sequences capable of directing the polypeptide to a cellular compartment or secreting it into the medium may be added to the coding sequence of the polynucleotide of the invention and are well known in the art. The leader sequence(s) is (are) assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein, or a portion thereof, into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an C- or N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (In-vitrogene), or pSPORT1

(GIBCO BRL).

Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences.

Furthermore, the present invention relates to a host comprising the polynucleotide or vector of the present invention.

Said host may be a prokaryotic or eukaryotic cell. The polynucleotide or vector of the invention which is present in the host cell may either be integrated into the genome of the host cell or it may be maintained extrachromosomally.

The host cell can be any prokaryotic or eukaryotic cell, such as a bacterial, insect, fungal, plant, animal or human cell. Preferred fungal cells are, for example, those of the genus Saccharomyces, in particular those of the species S. cerevisiae. The term "prokaryotic" is meant to include all bacteria which can be transformed or transfected with a polynucleotide or vector of the present invention for the expression of the antagonist of the present invention. Prokaryotic hosts may include gram negative as well as gram positive bacteria such as, for example, E. coli, S. typhimurium, Serratia marcescens and Bacillus subtilis. The term "eukaryotic" is meant to include yeast, higher plant, insect and preferably mammalian cells. Depending upon the host employed in a recombinant production procedure, the antagonist encoded by the polynucleotide of the present invention may or may not be post-translationally modified. A polynucleotide of the invention can be used to transform or transfect the host using any of the techniques commonly known to those of ordinary skill in the art. Furthermore, methods for preparing fused, operably linked genes and expressing them in, e.g., mammalian cells and bacteria are well-known in the art (e.g. Sambrook et al., Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989). The genetic constructs and methods described therein can be utilized for expression of the antagonist of the present invention in eukaryotic or prokaryotic hosts. In general, expression vectors containing promoter sequences which facilitate the efficient transcription of the inserted polynucleotide are used in connection with the host. The expression vector typically contains an origin of

replication, a promoter, and a terminator, as well as specific genes which are capable of providing phenotypic selection of the transformed cells. Furthermore, transgenic animals, preferably mammals, comprising host cells of the invention may be used for the large scale production of the antagonist of the present invention.

The present invention also relates to a method for producing the antagonist of the present invention, said method comprising culturing the host of the present invention under conditions that cause the synthesis of said antagonist, and recovering said antagonist from the culture.
Depending on the specific construct and condition used, the antagonist may be recovered from the host cells, from the culture medium or from both.

The present invention further relates to an antagonist obtainable by the method of the present invention.

Alternatively, the antagonist of the present invention can be chemically synthesized according to methods well known in the art, e.g., solid phase synthesis with Fmoc or t-boc chemistry (see also , e.g., Rühmann, A., A. K. E. Köpke, F. M. Dautzenberg, and J. Spiess, *Proc Natl. Acad. Sci. USA* **93:**10609-10613, 1996).

In another embodiment the present invention relates to an antibody directed against the antagonist of the present invention.

In a further embodiment the present invention relates to an anti-idiotypic antibody directed against the antibody of the present invention.
The antibodies of the present invention may be monoclonal antibodies, polyclonal antibodies, single chain antibodies, humanized antibodies, or fragments thereof that specifically bind the antagonist of the present invention or the antibody directed against the antagonist of the present invention. Bispecific antibodies, synthetic antibodies, antibody fragments, such as Fab, Fv or scFv fragments etc., or chemically modified derivatives of any of these are also encompassed by the present invention. Monoclonal antibodies can be prepared, for example, by the techniques as originally described in Köhler and Milstein, Nature 256 (1975), 495, and Galfré, Meth. Enzymol.

73 (1981), 3, which comprise the fusion of mouse myeloma cells to spleen cells derived from immunized mammals with modifications developed by the art. Furthermore, antibodies or fragments thereof can be obtained by using methods which are described, e.g., in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988. The production of chimeric antibodies is described, for example, in WO89/09622. Methods for the production of humanized antibodies are described in, e.g., EP-A1 0 239 400 and WO90/07861 A further source of antibodies to be utilized in accordance with the present invention are so-called xenogenic antibodies. The general principle for the production of xenogenic antibodies such as human antibodies in mice is described in, e.g., WO 91/10741, WO 94/02602, WO 96/34096 and WO 96/33735. As discussed above, the antibodies of the invention may exist in a variety of forms besides complete antibodies; including, for example, Fv, Fab and F(ab)2, as well as in single chains; see e.g WO88/09344.

The present invention also relates to a pharmaceutical composition comprising the antagonist, the polynucleotide, the vector, the antibody and/or the anti-idiotypic antibody of the present invention and optionally a pharmaceutically acceptable carrier and/or diluent.

Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g., by intravenous, intraperitoneal, subcutaneous, intramuscular, topical or intradermal administration. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. The compositions of the invention may be administered locally or systemically. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene

glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Furthermore, the pharmaceutical composition of the invention may comprise further agents depending on the intended use of the pharmaceutical composition.

Furthermore, the present invention relates to a diagnostic composition comprising the antagonist, the polynucleotide, the vector, the antibody and/or the anti-idiotypic antibody of the present invention.

The present invention further relates to a kit comprising the antagonist, the polynucleotide, the vector, the antibody and/or the anti-idiotypic antibody of the present invention.
The components of the diagnostic composition and/or the kit of the invention may be packaged in containers such as vials, optionally in buffers and/or solutions. If appropriate, one or more of said components may be packaged in one and the same container. Additionally or alternatively, one or more of said components may be adsorbed to solid support such as, e.g., a nitrocellulose filter or nylon membrane, or to the well of a microtiter plate.

In another embodiment the present invention relates to the use of the antagonist, the polynucleotide, the vector, the antibody and/or the anti-idiotypic antibody of the present invention for the preparation of a pharmaceutical composition for preventing and/or treating a Corticotropin-Releasing Factor Receptor, type 2 (CRFR2)-associated disease.

In a preferred embodiment of the antagonist or the use of the present invention said CRFR2 is CRFR2α or CRFR2β.

In another preferred embodiment the use of the present invention is for preventing and/or treating affective disorders, gastric intestinal diseases, cardiopathic diseases, psychiatric diseases, preferably eating disorders, anxiety disorders or anorexia nervosa, and/or Alzheimer's disease.

The present invention also relates to the use of the antagonist, the polynucleotide, the vector, the antibody and/or the anti-idiotypic antibody of the present invention for the investigation of CRF receptor type-specific functions.

The documents cited herein are herewith incorporated by reference.

Abbreviations used throughout the description the figure legends, and the examples are as follows: IUPAC rules are used for the nomenclature of peptides including one letter codes for amino acids. AAA: amino acid analysis; ACTH: adrenocorticotropic hormone; ANOVA: one-way analysis of variance; astressin: {cyclo(30-33)[DPhe^{12}, Nle^{21,38}, Glu^{30}, Lys^{33}]h/rCRF$_{(12-41)}$}; BSA: bovine serum albumin; cAMP: adenosine 3', 5'-cyclic monophosphate; CRF: corticotropin-releasing factor (h = human, o = ovine, r = rat); CRFR: CRF receptor; DIEA *N,N*-diisopropylethylamine; DMF: dimethylformamide; Fmoc: 9-fluorenylmethoxycarbonyl; HBTU: *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; HEK: human embryonic kidney; HOAc: acetic acid; HOBt: 1-hydroxybenzotriazole; ^{125}I: ^{125}I-iodinated; MeCN: acetonitrile; MS: mass spectrometry; NMP: *N*-methylpyrrolidone-2; OAll: *O*-allyl; OAlloc: O-allyloxycarbonyl; $Pd^0[PPh_3]_4$: tetrakis-(triphenylphosphine)-palladium; RP-HPLC: reverse phase high-performance liquid chromatography; SAR: structure-activity relationship; Svg: sauvagine; TFA: trifluoroacetic acid; Ucn: urocortin.

The figures show:

Figure 1:

Comparison of the amino acid sequence of human/rat corticotropin-releasing factor (h/rCRF) with ovine corticotropin-releasing factor (oCRF), rat urocortin (rUcn), sauvagine (Svg), and astressin. Identical amino acids are shaded.

Figure 2:

Chemical cross-linking of [^{125}I-Tyr^{0}]oCRF or [^{125}I-Tyr^{0}]Svg to membrane homogenates of human embryonic kidney (HEK) 293 cells stably transfected with cDNA coding for rat CRF receptor, type 1 (rCRFR1) (lanes 1 and 2) or mouse CRF receptor, type 2β (mCRFR2β) (lanes 3 and 4), respectively. Fifty µg of total membrane protein was labeled with approximately 100,000 cpm of [^{125}I-Tyr^{0}]oCRF (lanes 1 and 2) and [^{125}I-Tyr^{0}]Svg (lanes 3 and 4), and incubated (37°C, 30 min) in the presence (lane 2 and 4) or absence (lane 1 and 3) of 2000 units PNGase.

Figure 3:

Displacement of [^{125}I-Tyr^{0}]oCRF (A) or [^{125}I-Tyr^{0}]Svg (B) bound to membrane homogenates of HEK 293 cells stably transfected with cDNA coding for rat CRF receptor, type 1 (rCRFR1) (A), or mouse CRF receptor type 2β (mCRFR2β) (B), by oCRF (compound **1**, ○), Svg (compound **2**, □), astressin (compound **3**, △), [DPhe^{12}, Nle^{21,38}]h/rCRF$_{(12-41)}$ (compound **5**, ▲), [DPhe^{12}]oCRF$_{(12-41)}$ (compound **6**, ●), [DPhe^{11}]rUcn$_{(11-40)}$ (compound **7**, ◆), and [DPhe^{11}, His^{12}]Svg$_{(11-40)}$ (compound **11**, ■). The error bars represent the SEM and are not shown when smaller than the symbol size.

Figure 4:

Potency of 1µM CRF antagonist (compounds **3-14**) to stimulate cAMP production in transfected HEK 293 cells (%IA, intrinsic activity) (A, B). Efficacy of CRF antagonists (c = 1µM for rCRFR1 and c = 10nM for mCRFR2β) to inhibit cAMP production in transfected HEK 293 cells stimulated by 1nM oCRF (compound **1** for rCRFR1) and 1nM Svg (compound **2** for mCRFR2β) (relative *in vitro* potency) (C, D). Data is the mean from 3-11 experiments. The error bars represent the SEM.

The examples illustrate the invention.

Example 1: Synthesis and analysis of peptides

Different truncated and conformationally constrained analogs of corticotropin-releasing factor (CRF) were synthesized on the basis of the amino acid sequences of human/rat CRF (h/rCRF), ovine CRF (oCRF), rat urocortin (rUcn), or sauvagine (Svg) (0.1 mmol scale) with Fmoc chemistry on TentaGel S RAM resin (Rapp, Tübingen, F.R.G.) with a model ABI 433A peptide synthesizer (Applied Biosystems). Comparison of the amino acid sequences of oCRF, rUcn and Svg with the sequence of h/rCRF reveals 45-83% amino acid identity. The CRF ligands mentioned share high amino acid identity at the N-terminus (47%) stretching from amino acid 2-20 (h/rCRF and oCRF) and 1-19 (rUcn and Svg), but little at the C-terminus (14%) of the peptides stretching from amino acid 21-41 and 20-40, respectively (Fig. 1). It was assumed that the ligand receptor interactions of the truncated forms of the CRF peptides ranging from amino acid 11-40 (rUcn and Svg) or 12-41 (h/rCRF and oCRF) acted differently than the full-length CRF peptides on CRFR1 or CRFR2 (Dautzenberg, F. M., K. Dietrich, M. R. Palchaudhuri, and J. Spiess, *J. Neurochem.* **69**:1640-1649, 1997; Vaughan, J., C. Donaldson, J. Bittencourt, M. H. Perrin, K. Lewis, S. Sutton, R. Chan, A. V. Turnbull, D. Lovejoy, C. Rivier, J. Rivier, P. E. Sawchenko, and W. Vale, *Nature (London)* **378**:287-292, 1995; Donaldson, C. J., S. W. Sutton, M. H. Perrin, A. Z. Corrigan, K. A. Lewis, J. E. Rivier, J. M. Vaughan, and W. W Vale, *Endocrinology* **137**:2167-2170, 1996; Gottowik, J., V. Goetschy, S. Henriot, E. Kitas, B. Fluhman, R. G. Clerc, J. L. Moreau, F. J. Monsma, and G. J. Kilpatrick, *Neuropharmacol.* **36**:1439-1446, 1997).

For the synthesis of the cyclized CRF analogs, astressin and cyclo(29-32)[DPhe^{11}, Glu^{29}, Lys^{32}]rUcn$_{(11-40)}$, the amino acid derivatives Fmoc-L-Glu(OAll)-OH and Fmoc-L-Lys(Alloc)-OH (PerSeptive Biosystems GmbH, Hamburg, F.R.G.) were used. The side-chain-protected peptides were reacted with $Pd^0[PPh_3]_4$ in HOAc/N-methylaniline/dichloromethane (v/v; 2:1:40) for three hours and then cyclized with HOBt/HBTU in DMF and DIEA in NMP for eight hours. After removal of the N-terminal Fmoc group with piperidine in NMP, the peptides were cleaved from the resin under standard conditions. The crude peptides were purified by preparative reverse phase high-performance liquid chromatography (RP-HPLC) performed on a Waters Prep Nova-Pak HR C_{18} silica gel column (5 x 30 cm, 6-µm particle size, 6-nm pore size) with a mixture of aqueous 0.1% trifluoroacetic acid and MeCN (see Table 5). The mass spectra of the purified peptides were measured with a plasma desorption mass

spectrometer (Biolon 20, Uppsala). Amino acid analysis (AAA) was performed after hydrolysis of peptides (6 N HCl, 3 hr, 150°C) with a Beckman High Performance Analyzer System 6300 (Beckman, San Ramon).

Example 2: Binding of CRF agonists and antagonists to rCRFR1

CRF agonists and antagonists were tested in an *in vitro* assay for their ability to displace [^{125}I-Tyr^{0}]oCRF or [^{125}I-Tyr^{0}]Svg from membranes of HEK-rCRFR1 cells (Rühmann, A., A. K. E. Köpke, F. M. Dautzenberg, and J. Spiess, *Proc. Natl. Acad. Sci. USA* **93:**10609-10613, 1996) or HEK-mCRFR2β cells (Kishimoto, T., R. V. Pearse II, C. R. Lin, and M. G. Rosenfeld, *Proc. Natl Acad. Sci. USA* **92:**1108-1112, 1995). Binding assays were performed in 96-well MultiScreen plates (Millipore, Eschborn, Germany) with GF/B filters (pore size 1.0 µm). Fifty-microliters of membrane suspension (25 µg of protein from HEK-rCRFR1 cells; 50 µg of protein from HEK-mCRFR2β cells) was added to a plate containing CRF peptides (c = 0-1 µM) and 50,000 cpm of either [^{125}I-Tyr^{0}]oCRF (specific activity 81.4 TBq/mmol, 68.25 pM, DuPont NEN, Boston) for the analysis of rCRFR1 or [^{125}I-Tyr^{0}]Svg (specific activity 81.4 TBq/mmol, 68.25 pM, DuPont NEN, Boston) for the analysis of mCRFR2β in 100 µl incubation buffer (50 mM Tris/Cl, 5 mM $MgCl_2$, 2 mM EGTA, 100,000 kallikrein inhibitor units per liter of Trasylol (Bayer, Leverkusen), 1 mM dithiothreitol, 1 mg/ml BSA, pH 7.4). After incubation (60 min, 23°C), membrane suspension was aspirated through the plate, followed by two washes with assay buffer (0.2 ml, 23°). Radioactivity of the punched filters was measured with a 1470 WIZARD automatic gamma counter (Berthold, Hannover). Specific binding of [^{125}I-Tyr^{0}]oCRF or [^{125}I-Tyr^{0}]Svg to membranes of transfected cells was calculated by substration of unspecific binding found in the presence of 1 µM of nonlabeled ligand from total binding. Data analysis was achieved with the nonlinear curve fitting program LIGAND. Statistical analysis was performed with ANOVA, and significant differences between groups were determined by post hoc comparison using the Dunn test.

Example 3: Chemical cross-linking with [^{125}I-Tyr^{0}]oCRF or [^{125}I-Tyr^{0}]Svg

Chemical cross-linking was carried out in 1.5 ml polypropylene tubes (Sigma, Deisenhofen, Germany) as for the binding assay except that no BSA was used.

Samples (50 µg and 100 µg of protein from membrane fractions of HEK-rCRFR1 cells and HEK-mCRFR2β cells, respectively) were reacted with 10 µl of disuccinimidyl suberate (1.5 mM in dimethylsulfoxide, 23°C, 20 min) after incubation with ligand (V = 300 µl, 100,000 cpm, 1 hr, 23°C). The reaction was terminated by the addition of 1.0 ml of ice-cold buffer (10 mM Tris/Cl, 1 mM EDTA, pH 7.0, 4°C). In some experiments, the chemically cross-linked receptor was deglycosylated with PNGase (New England Biolabs, Schwalbach). Samples were then heated (100°C, 5 min) and subjected to SDS PAGE. Autoradiography was carried out on a BAS-IP NP 2040P imaging plate. Radioactivity was monitored with a Fujix BAS 2000 scanner (Raytest, Straubenhardt). Gel documentation was accomplished with the program TINA (Raytest).

Example 4: Determination of cAMP stimulation

HEK-rCRFR1 cells or HEK-mCRFR2β cells were incubated with different CRF agonists in the presence or absence of 1 µM or 10 nM antagonist, or CRF antagonist (c = 1 µM) alone. After removal of the medium, cells were lyzed with aqueous 6% trichloroacetic acid (5 min, 100°C) (Rühmann, A., A. K. E. Köpke, F. M. Dautzenberg, and J. Spiess, *Proc. Natl. Acad. Sci. USA* 93:10609-10613, 1996). The cell lysates were stored at -70°C until assayed with a RIA kit (Amersham, Little Chalfont). Data analysis was achieved with the sigmoidal dose-response curve fitting program ALLFIT. Statistical significance was determined across groups with ANOVA, and significant differences between groups were determined by post hoc comparison using the Dunn test.

Example 5: Displacement of [^{125}I-Tyr^{0}]oCRF or [^{125}I-Tyr^{0}]Svg from recombinant rCRFR1 or mCRFR2β by CRF analogs

Membrane homogenates of human embryonic kidney (HEK) 293 cells stably transfected with cDNA coding for rat CRF receptor, type 1 (rCRFR1) or mouse CRF receptor, type 2β (mCRFR2β) were prepared according to standard protocols (see, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd. Edition, Cold Spring Habor Laboratory Press, Cold Spring Habor, NY, 1989; Ausubel et al., Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, NY, 1989). The specific binding of [^{125}I-Tyr^{0}]oCRF to membranes of HEK-rCRFR1 cells

was found to be 93% when the radioligand was displaced by oCRF. In analogous displacement experiments with Svg, the specific binding of [^{125}I-Tyr^{0}]Svg to membranes of HEK-mCRFR2β cells was determined to be 94%. No specific binding of the two radioactively labeled CRF analogs to membranes of nontransfected HEK 293 cells could be observed. These data could be confirmed when [^{125}I-Tyr^{0}]oCRF and [^{125}I-Tyr^{0}]Svg were chemically cross-linked to rCRFR1 and mCRFR2β, respectively. The molecular weight of both cross-linked receptors was 66,000. After deglycosylation with PNGase, molecular weights of 43,000 and 41,000 were found for crosslinked mCRF2β and rCRFR1, respectively (Fig. 2), which are in agreement with the molecular weight predicted on the basis of DNA data (Perrin, M. H., C. J. Donaldson, R. Chen, K. A. Lewis, and W. Vale, *Endocrinology* 133:3058-3061, 1993; Kishimoto, T., R. V. Pearse II, C. R. Lin, and M. G. Rosenfeld, *Proc. Natl. Acad. Sci. USA* **92**:1108-1112, 1995). The difference of 2,000 between the molecular weights of rCRFR1 and mCRFR2β is probably due to the longer amino acid sequence of mCRFR2β. No chemical cross-links could be observed with either radioligand to nontransfected HEK 293 cells.

As expected for the CRF peptide agonists (Dautzenberg, F. M., K. Dietrich, M. R. Palchaudhuri, and J. Spiess, *J. Neurochem.* **69**:1640-1649, 1997; Donaldson, C. J., S. W. Sutton, M. H. Perrin, A. Z. Corrigan, K. A. Lewis, J. E. Rivier, J. M. Vaughan, and W. W Vale, *Endocrinology* **137**:2167-2170, 1996; Giottowik, J., V. Goetschy, S. Henriot, E. Kitas, B. Fluhrman, R. G. Clerc, J. L. Moreau, F. J. Monsma, and G. J. Kilpatrick, *Neuropharmacol.* **36**:1439-1446, 1997), oCRF and Svg exhibited similar high-affinity binding to rCRFR1 (oCRF: K_d = 0.6 ± 0.1 nM, Svg: K_d = 0.7 ± 0.1 nM), but differed significantly in their binding to mCRFR2β (oCRF: K_d = 162.4 ± 37.6 nM, Svg: K_d = 4.5 ± 0.6 nM) (Table 1, Fig. 3).

For the binding of the CRF antagonists, (α-helical CRF$_{(9-41)}$ and [DPhe^{12}, Nle^{21,38}]h/rCRF$_{(12-41)}$ described earlier (Gulyas, J., C. Rivier, M. Perrin, S. C. Koerber, S. Sutton, A. Corrigan, S. L. Lahrichi, A. G. Craig, W. Vale, and J. Rivier, *Proc. Natl. Acad. Sci. USA* **92**:10575-10579, 1995) to rCRFR1, K_c values of 60.3 ± 10.6 nM and 46.4 ± 9.4 nM were obtained, respectively. The antagonist astressin (Gulyas, J., C. Rivier, M. Perrin, S. C. Koerber, S. Sutton, A. Corrigan, S. L. Lahrichi, A. G. Craig, W.

Vale, and J. Rivier, *Proc. Natl. Acad. Sci. USA* **92:**10575-10579, 1995), cyclo(30-33)[DPhe^{12}, Nle^{21,38}, Glu^{30}, Lys^{33}]h/rCRF$_{(12-41)}$, was found to bind nonselectively with similar affinity to rCRFR1 (K_d = 5.7 ± 1.6 nM) and mCRFR2β (K_d = 4.0 ± 2.3 nM), whereas α-helical CRF$_{(9-41)}$ and [DPhe^{12}, Nle^{21,38}]h/rCRF$_{(12-41)}$ showed moderate selectivity for binding to mCRFR2β with K_d values of 6.4 ± 0.9 nM and 17.7 ± 2.2 nM, respectively (Table 1). [DPhe^{12}]oCRF$_{(12-41)}$ based on the amino acid sequence of oCRF showed low-affinity binding to rCRFR1 and mCRFR2β with K_d values of 290.2 ± 74.7 nM and 153.8 ± 26.8 nM, respectively (Table 1, Fig. 3).

The truncated Ucn analogs [DPhe^{11}]rUcn$_{(11-40)}$, [DPhe^{11}, Glu^{12}]rUcn$_{(11-40)}$, [DLeu^{11}, Glu^{12}]rUcn$_{(11-40)}$, and cyclo(29-32)[DPhe^{11}, Glu^{29}, Lys^{32}]rUcn$_{(11-40)}$ exhibited moderate binding affinity to rCRFR1 with K_d values of 33.0 ± 5.9 nM, 68.2 ± 20.5 nM, 91.1 ± 21.2 nM, and 47.1 ± 8.9 nM, respectively, and revealed little preference for binding to mCRFR2β with K_d values of 5.2 ± 1.5 nM, 9.5 ± 2.0 nM, 27.9 ± 3.4 nM, and 22.4 ± 4.6 nM, respectively (Table 1, Fig. 3).

The Svg-derived peptides [DLeu^{11}]Svg$_{(11-40)}$, Svg$_{(11-40)}$, [DPhe^{11}]Svg$_{(11-40)}$, and [DPhe^{11}, His^{12}]Svg$_{(11-40)}$ showed low-affinity binding to rCRFR1 with K_d values of 1670.0 ± 500.0 nM, 831.2 ± 668.8 nM, 237.3 ± 27.7 nM, and 153.6 ± 33.5 nM, respectively, but high-affinity binding to mCRFR2β with K_d values of 20.9 ± 4.1 nM, 15.4 ± 2.5 nM, 3.5 ± 0.2 nM, and 1.4 ± 0.4 nM, respectively (Table 1). Thus, [DPhe^{11}, His^{12}]Svg$_{(11-40)}$, [DLeu^{11}]Svg$_{(11-40)}$, [DPhe^{11}]Svg$_{(11-40)}$, and Svg$_{(11-40)}$ bound with a 77-, 56-, 47-, and 38-fold higher selectivity to mCRFR2β than astressin, respectively.

ANOVA indicated statistically significant differences in high-affinity binding, $F(13,41) = 13.17$ $p = 0.0001$, of the CRF peptides (compounds **1-14**) to cell membranes of HEK-rCRFR1 cells (Table 1). Post hoc comparison demonstrated a significantly lower binding affinity of [DLeu^{11}]Svg$_{(11-40)}$ (compound **13**) and Svg$_{(11-40)}$ (compound **14**) to cell membranes when compared to compounds **1-5**, and **7-11** ($p = 0.0001$) (Table 1). Statistically significant differences in high-affinity binding, $F(13,36) = 20.34$ $p = 0.0001$, of oCRF (compound **1**) and [DPhe^{12}]oCRF$_{(12-41)}$ (compound **6**) to cell membranes of HEK-mCRFR2β cells were observed. Post hoc

comparison demonstrated a significantly lower binding affinity of oCRF (compound **1**) and [DPhe^{12}]oCRF$_{(12-41)}$ (compound **6**) to cell membranes when compared to compounds **2-5** and compounds **7-14** (p = 0.0001) (Table 1).

Thus, in comparison to astressin, the most potent CRF antagonist described to date (Gulyas, J., C. Rivier, M. Perrin, S. C. Koerber, S. Sutton, A. Corrigan, S. L. Lahrichi, A. G. Craig, W. Vale, and J. Rivier, *Proc. Natl. Acad. Sci. USA* **92**:10575-10579, 1995), the intrinsic activity of [DPhe^{11}, His^{12}]Svg$_{(11-40)}$ was not significantly different in experiments with HEK-rCRFR1 or HEK-mCRFR2β cells. However, the inhibitory potency of [DPhe^{11}, His^{12}]Svg$_{(11-40)}$ towards rCRFR1 was found to be 15% of the potency of astressin. This difference was determined to be significant. In contrast, no significant difference between the inhibitory potencies of astressin and [DPhe^{11}, His^{12}]Svg$_{(11-40)}$ was observed, when HEK-mCRF2β cells were tested (Table 2). The difference between astressin and [DPhe^{11}, His^{12}]Svg$_{(11-40)}$ was even more pronounced in binding experiments (Table 1) which demonstrated that [DPhe^{11}, His^{12}]Svg$_{(11-40)}$ exhibited in contrast to astressin selective binding to mCRFR2β. On the basis of ligand comparisons, [DPhe^{11}, His^{12}]Svg$_{(11-40)}$ was thus demonstrated to be a selective, mCRFR2β-directed CRF antagonist with low intrinsic activities directed towards rCRFR1 and mCRFR2β.

In contrast to CRFR2, mammalian CRFR1 has been reported to be nonselective for CRF and CRF-like peptides including the structurally related 40-amino acid peptides Svg and Ucn (Vita, N., P. Laurent, S. Lefort, P. Chalon, J.-M. Lelias, M. Kaghad, G. Le Fur, D. Caput, and P. Ferrara, *FEBS Lett.* **335**:1-5, 1993; Dautzenberg, F. M., K. Dietrich, M. R. Palchaudhuri, and J. Spiess, *J. Neurochem.* **69**:1640-1649, 1997; Vaughan, J., C. Donaldson, J. Bittencourt, M. H. Perrin K. Lewis, S. Sutton, R. Chan, A. V. Turnbull, D. Lovejoy, C. Rivier, J. Rivier, P. E. Sawchenko, and W. Vale, *Nature (London)* **378**:287-292, 1995; Fisher, L., C. Rivier, J. Rivier, and M. Brown, *Endocrinology* **129**:1312-1316, 1991; Donaldson, C. J., S. W. Sutton, M. H. Perrin, A. Z. Corrigan, K. A. Lewis, J. E. Rivier, J. M. Vaughan, and W. W Vale, *Endocrinology* **137**:2167-2170, 1996). Experimental data available thus far do not show significant pharmacological differences between mammalian CRFR2α and CRFR2β (Fisher, L., C. Rivier, J. Rivier, and M. Brown, *Endocrinology* **129**:1312-1316, 1991; Donaldson, C.

J., S. W. Sutton, M. H. Perrin, A. Z. Corrigan, K. A. Lewis, J. E. Rivier, J. M. Vaughan, and W. W Vale, *Endocrinology* **137**:2167-2170 1996). On this basis, it is expected that [DPhe^{11}, His^{12}]Svg$_{(11-40)}$ inhibits CRFR2α similarly as CRFR2β. Indeed, results obtained from in vivo experiments with BALB/c mice showed that [DPhe^{11}, His^{12}]Svg$_{(11-40)}$ inhibited CRF-mediated behavioral effects in the lateral septum (J. Radulovic, A. Rühmann, T. Liepold and J. Spiess, J. Neurosci. 19 (1999), 5016-5025; D.T. Chalmers, T.W. Lovenberg and E. B. DeSouza, J. Neurosci. 15 (1995), 6340-6350) an area in the brain which exclusively expresses CRF2α-mRNA.

Example 6: cAMP stimulation

The peptide agonists oCRF and Svg exhibited high potency to increase cAMP accumulation in HEK-rCRFR1 cells with EC_{50} values of 0.41 ± 0.08 nM and 0.19 ± 0.05 nM, respectively. but differed significantly in their potencies to stimulate cAMP production in HEK-mCRFR2β cells with EC_{50} values of 11.79 ± 1.96 nM and 0.23 ± 0.05 nM, respectively.

The CRF antagonists (compounds **3-14**, Table 2, Fig. 4) mentioned above were tested for their ability to enhance or inhibit oCRF- and Svg-stimulated cAMP production in transfected HEK 293 cells expressing rCRFR1 (HEK-rCRFR1 cells) and mCRFR2β (HEK-mCRFR2β cells), respectively. Intrinsic activities were measured at peptide concentrations generating maximal formation of cAMP in HEK-rCRFR1 or HEK-mCRFR2β cells. The intrinsic activities of CRF antagonists tested by their effect at a concentration of 1 μM on HEK-rCRFR1 cells ranged from 4-23% of the intrinsic activity of oCRF (compound **1**) tested at a concentration of 1 nM. The intrinsic activities of the antagonists tested under equivalent conditions with HEK-mCRFR2β cells were 0.4-0.9% of the intrinsic activity of Svg (compound **2**) (Table 2, Fig. 4).

ANOVA indicated statistically significant differences in intrinsic activity, $F(12,50) = 11.68$, $p = 0.0001$, of compounds **3-14** to stimulate cAMP production in HEK-rCRFR1 cells. Post hoc comparison demonstrated a significantly higher intrinsic activity of compounds **8**, **9**, and **13** when compared to compounds **5**, **6**, **7**, **11**, and **12** ($p = 0.001$). Compounds **4** and **14** also significantly increased cAMP production in HEK-rCRFR1 cells when compared to the basal level of cAMP in the same cells ($p = 0.001$) (Table 2). ANOVA indicated no statistically significant differences in

intrinsic activity of compounds **3-14** to stimulate cAMP production in HEK-mCRFR2β cells. Compound **11** exhibited the lowest intrinsic activity of all tested CRF antagonists in experiments with either recombinant system, HEK-rCRFR1 cells and HEK-mCRFR2β cells (Table 2).

The rank order of potencies for the CRF related peptide antagonists to suppress oCRF-induced cAMP-accumulation in HEK-rCRFR1 cells was as follows: astressin (compound **3**) and [DPhe^{11}]rUcn$_{(11-40)}$ (compound **7**) > [DPhe^{12}, Nle^{21,38}]h/rCRF$_{(12-41)}$ (compound **5**) and [DPhe^{11}, Glu^{12}]rUcn$_{(11-40)}$ (compound **8**) > [DLeu^{11}, Glu^{12}]rUcn$_{(11-40)}$ (compound **9**) and α-helical CRF$_{(9-41)}$ (compound **4**) > [DPhe^{11}, His^{12}]Svg$_{(11-40)}$ (compound **11**), [DPhe^{11}]Svg$_{(11-40)}$ (compound **12**), Svg$_{(11-40)}$ (compound **14**), and cyclo(29-32)[DPhe^{11}, Glu^{29}, Lys^{32}]rUcn$_{(11-40)}$ (compound **10**) > [DPhe^{12}]oCRF$_{(12-41)}$ (compound **6**) and [DLeu^{11}]Svg$_{(11-40)}$ (compound **13**). In contrast, the following pharmacological profile was obtained for the inhibition of Svg-stimulated cAMP production in HEK-mCRFR2β cells by CRF antagonists: [DPhe^{11}, His^{12}]Svg$_{(11-40)}$ (compound **11**) > astressin (compound **3**), [DPhe^{11}]rUcn$_{(11-40)}$ (compound **7**), [DPhe^{11}, Glu^{12}]rUcn$_{(11-40)}$ (compound **8**), and α-helical CRF$_{(9-41)}$ (compound **4**) > [DPhe^{11}]Svg$_{(11-40)}$ (compound **12**), [DLeu^{11}]Svg$_{(11-40)}$ (compound **13**), and Svg$_{(11-40)}$ (compound **14**) > [DLeu^{11}, Glu^{12}]rUcn$_{(11-40)}$ (compound **9**) > [DPhe^{12}, Nle^{21,38}]h/rCRF$_{(12-41)}$ (compound **5**), cyclo(29-32)[DPhe^{11}, Glu^{29}, Lys^{32}]rUcn$_{(11-40)}$ (compound **10**), and [DPhe^{12}]oCRF$_{(12-41)}$ (compound **6**).

ANOVA indicated statistically significant differences in potency, $F(12,39) = 7.93$, $p = 0.0001$, of compounds **3-14** to inhibit oCRF-stimulated cAMP production in HEK-rCRFR1 cells. Post hoc comparison demonstrated a significantly higher potency of compounds **3** and **7** when compared to compounds **6** ($p = 0.0001$) and compounds **10-14** ($p = 0.0001$). A significantly higher potency was also observed for compound **5** when compared to compounds **6** ($p = 0.001$) and **13** ($p = 0.0001$).

ANOVA indicated statistically significant differences in potency, $F(12,65) = 6.34$, $p = 0.0001$, of compounds **3-14** to inhibit Svg-stimulated cAMP production in HEK-mCRFR2β cells. Post hoc comparison demonstrated a significantly higher potency of compound **11** when compared to compounds **2** and **4** ($p = 0.001$) or compounds **5**, **6**,

9, 10, and 13 (p = 0.0001).

Example 7: Synthesis of 4-(1-azi-2,2,2-trifluoroethyl)benzoic acid

4-(1-azi-2,2,2-trifluoroethyl)benzoic acid was synthesized as described in PCT/EP96/05011.

Example 8: Analysis of CRFR2-selective antagoinsts suitable for radioactive labelling and photoaffinity labelling experiments

Corticotropin-releasing factor (CRF) receptor, type 2β (CRFR2β)-selective antagonists based on the truncated amino acid sequence of sauvagine (Svg) were synthesized and characterized. The N-terminal amino acid Leu^{11} in Svg (11-40) was substituted by either tyrosine or a phenyldiazirine, the 4-(1-azi-2,2,2-trifluoroethyl)benzoyl (ATB) group, for radioactive labelling or photoaffinity labelling experiments, respectively. To further increase the binding affinity of the ligands to membrane homogenates of human embryonic kidney (HEK) cells permanently transfected with cDNA coding for rat CRFR1 (rCRFR1) or mouse CRFR2β (mCRFR2β), Glu^{12} was substituted by histidine.

The binding affinity of the compounds to rCRFR1 and mCRFR2β and the potency of the ligands to produce cAMP accumulation (agonistic activity) or inhibit oCRF-(rCRFR1) or Svg-(mCRFR2β) stimulated cAMP production (antagonistic activity) in transfected cells were compared with the binding affinity and potency of antisauvagine-30 {[DPhe^{11}, His^{12}]Svg(11-40)} (compound 11), astressin {cyclo(30-33)[DPhe^{12}, Nle^{21,38}, Glu^{30}, Lys^{33}]h/rCRF(12-41) (compound 3) and the photoactivatable astressin analogs ATB-cyclo(30-33)[Nle^{21,38}, Glu^{30}, Ala^{32}, Lys^{33}]h/rCRF(13-41) (compound 15) and ATB-cyclo(30-33)[Nle^{21,38}, Glu^{30}, Tyr^{32}, Lys^{33}]h/rCRF(13-41) (compound 16).

Substitution of DPhe^{11} by the phenyldiazirine moiety in ATB-[His^{12}]Svg(12-40) (compound 17) did not significantly change the binding affinity, ligand selectivity, and potency of this ligand to CRFR1 and mCRFR2β when compared with antisauvagine-30 (compound 11) (Table 3 and 4). Similar results could be observed for ATB-cyclo(30-33)[(Nle^{21,38}, Glu^{30}, Ala^{32}, Lys^{33}]h/rCRF(13-41) (compound 15) when compared with astressin (compound 3)(Table 3 and 4).

Substitution of DPhe^{11} by tyrosine in [Tyr^{11}, His^{12}]Svg(11-40) (compound 18) or

[Tyr^{11}]Svg(11-40) (compound 19) decreased the binding affinity of these compounds to rCRFR1 or mCRFR2β by 1.4 to 5.9-fold when compared to antisauvagine-30 (compound 11) or [DPhe^{11}]Svg(11-40) (compound 12), respectively, without changing preferable binding to mCRFR2β. Further N-terminal truncation of the amino acid sequence Svg(11-40) by one amino acid and substitution of Glu^{12} by tyrosine completely abolished high-affinity binding of [Tyr^{12}]Svg(12-40) (compound 20) to mCRFR2β (Table 3).

TABLE I

Binding constants (K_d, [nM]) found for different CRF-related agonists and antagonists when bound to recombinant rCRFR1 with [^{125}I Tyr^0]oCRF as competitive ligand or recombinant mCRFR2β with [^{125}I-Tyr^0]Svg as competitive ligand.

No.	peptides	[^{125}I-Tyr^0]oCRF $K_{d(rCRFR1)}$, [nM]	[^{125}I-Tyr^0]Svg $K_{d(mCRFR2\beta)}$, [nM]	*P*	Selectivity[e]
1	oCRF	0.6 ± 0.1	162.4 ± 37.6[c]	[*]	0.002
2	Svg	0.7 ± 0.1	4.5 ± 0.6	[**]	0.109
3	astressin	5.7 ± 1.6	4.0 ± 2.3	N.S.	1.00
4	α-helical CRF$_{(9-41)}$	60.3 ± 10.6	6.4 ± 0.9	[**]	6.61
5	[DPhe^{12}, Nle^{21,38}]h/rCRF$_{(12-41)}$	46.4 ± 9.4	17.7 ± 2.2	[*]	1.84
6	[DPhe^{12}]oCRF$_{(12-41)}$	290.2 ± 74.7	153.8 ± 26.8[d]	N.S.	1.32
7	[DPhe^{11}]rUcn$_{(11-40)}$	33.0 ± 5.9	5.2 ± 1.5	[**]	4.45
8	[DPhe^{11}, Glu^{12}]rUcn$_{(11-40)}$	68.2 ± 20.5	9.5 ± 2.0	[*]	5.04
9	[DLeu^{11}, Glu^{12}]rUcn$_{(11-40)}$	91.1 ± 21.2	27.9 ± 3.4	[*]	2.39
10	cyclo(29-32) [DPhe^{11}, Glu^{29}, Lys^{32}]rUcn$_{(11-40)}$	47.1 ± 8.9	22.4 ± 4.6	N.S.	1.48
11	[DPhe^{11}, His^{12}]Svg$_{(11-40)}$	153.6 ± 33.5	1.4 ± 0.4	[**]	76.99
12	[DPhe^{11}]Svg$_{(11-40)}$	237.3 ± 27.7	3.5 ± 0.2	[*]	47.58
13	[DLeu^{11}]Svg$_{(11-40)}$	1670.0 ± 500.0[a]	20.9 ± 4.1	[**]	56.07
14	Svg$_{(11-40)}$	831.2 ± 668.8[b]	15.4 ± 2.5	N.S.	37.88

Statistically significant differences between the K_d values of the peptides: [a], $p \leq 0.0001$ vs. 1-12, 14. [b], $p \leq 0.0001$ vs. 1-5, 7-11, 13. [c],[d], $p \leq 0.0001$ vs. 2-5, 7-14. *P* values of unpaired Student's *t* test obtained by comparing the K_d value with the K_d value of each peptide. ***, $p \leq 0.001$. **, $0.01 \geq p > 0.001$. *, $0.05 \geq p > 0.01$. N.S., not significant. [e], Selectivity was calculated according to the equation: Selectivity = $K_{d(rCRFR1)}$ (agonist/antagonist)/$K_{d(rCRFR1)}$ (astressin) x $K_{d(mCRFR2\beta)}$ (astressin)/$K_{d(mCRFR2\beta)}$ (agonist/antagonist).

TABLE 2

Potency of 1 μM CRF antagonist to stimulate cAMP production in transfected HEK 293 cells (%IA, intrinsic activity). Efficacy of 1 μM (rCRFR1) or 10 nM (mCRFR2β) CRF antagonist to inhibit cAMP production stimulated by oCRF (rCRFR1) or Svg (mCRFR2β) (1 nM) in transfected HEK 293 cells (relative *in vitro* potency).

No.	peptides	rCRFR1 %IA	relative *in vitro* potency	mCRFR2β %IA	relative *in vitro* potency
1	oCRF	1.00	1.00	-	-
2	Svg	-	-	1.00	1.00
3	astressin	0.10 ± 0.02	0.11 ± 0.03[f]	0.004 ± 0.001	0.57 ± 0.04
4	α-helical $CRF_{(9-41)}$	0.16 ± 0.02[a]	0.58 ± 0.05	0.007 ± 0.004	0.67 ± 0.02
5	[$DPhe^{12}$, $Nle^{21,38}$]h/rCRF$_{(12-41)}$	0.06 ± 0.01	0.33 ± 0.08[h,i]	0.007 ± 0.002	0.87 ± 0.08[i]
6	[$DPhe^{12}$]oCRF$_{(12-41)}$	0.05 ± 0.01	0.89 ± 0.07	0.008 ± 0.003	0.92 ± 0.07[k]
7	[$DPhe^{11}$]rUcn$_{(11-40)}$	0.07 ± 0.01	0.18 ± 0.03[g]	0.011 ± 0.003	0.57 ± 0.06
8	[$DPhe^{11}$, Glu^{12}]rUcn$_{(11-40)}$	0.22 ± 0.05[b]	0.42 ± 0.11	0.004 ± 0.001	0.61 ± 0.02
9	[$DLeu^{11}$, Glu^{12}]rUcn$_{(11-40)}$	0.23 ± 0.04[c]	0.56 ± 0.05	0.009 ± 0.002	0.81 ± 0.06
10	cyclo(29-32)[$DPhe^{11}$, Glu^{29}, Lys^{32}] rUcn$_{(11-40)}$	0.10 ± 0.06	0.78 ± 0.10	0.005 ± 0.001	0.89 ± 0.01
11	[$DPhe^{11}$, His^{12}]Svg$_{(11-40)}$	0.04 ± 0.01	0.73 ± 0.07	0.004 ± 0.001	0.42 ± 0.02[l,m]
12	[$DPhe^{11}$]Svg$_{(11-40)}$	0.07 ± 0.01	0.75 ± 0.13	0.005 ± 0.001	0.71 ± 0.04
13	[$DLeu^{11}$]Svg$_{(11-40)}$	0.22 ± 0.04[d]	0.92 ± 0.16	0.008 ± 0.002	0.75 ± 0.06
14	Svg$_{(11-40)}$	0.14 ± 0.02[e]	0.76 ± 0.16	0.005 ± 0.001	0.76 ± 0.02
	basal	0.01 ± 0.003		0.004 ± 0.001	

Statistically significant differences between the intrinsic activities of the peptides: [a], $0.001 \geq p > 0.0001$ vs. 15. [b,c,d], $p \leq 0.0001$ vs. 5, 6, 7, 11, 12, 15. [e], $p \leq 0.0001$ vs. 15. Statistically significant differences between the relative potencies of the peptides: [f,g], $p \leq 0.0001$ vs. 6, 10-14. [h], $0.001 \geq p > 0.0001$ vs. 6. [i], $p \leq 0.0001$ vs. 13. [j], $0.001 \geq p > 0.0001$ vs. 3, 7. [k], $p \leq 0.0001$ vs. 7. [l], $p \leq 0.0001$ vs. 5, 6, 9, 10, 13. [m], $0.001 \geq p > 0.0001$ vs. 2, 4.

TABLE 3

Binding constants (K_d, [nM]) of different CRF-related agonists and antagonists displacing [^{125}I-Tyr^0]oCRF from recombinant rCRFR1 or [^{125}I-Tyr^0]Svg from recombinant mCRFR2β.

No.	peptides	[^{125}I-Tyr^0]oCRF $K_{d(rCRFR1)}$, [nM]	[^{125}I-Tyr^0]Svg $K_{d(mCRFR2\beta)}$, [nM]	Selectivity
1	oCRF	0.6 ± 0.1	162.4 ± 37.6	0.002
2	Svg	0.7 ± 0.1	4.5 ± 0.6	0.109
3	astressin[a]	5.7 ± 1.6	4.0 ± 2.3	1.00
11	[DPhe^{11}, His^{12}]Svg$_{(11-40)}$	153.6 ± 33.5	1.4 ± 0.4	76.99
12	[DPhe^{11}]Svg$_{(11-40)}$	237.3 ± 27.7	3.5 ± 0.2	47.58
13	[DLeu^{11}]Svg$_{(11-40)}$	1670.0 ± 500.0	20.9 ± 4.1	56.07
14	Svg$_{(11-40)}$	831.2 ± 668.8	15.4 ± 2.5	37.88
15	ATB-[Ala^{32}]astressin[b,c]	5.3 ± 1.3	2.6 ± 1.0	1.43
16	ATB-[Tyr^{32}]astressin[d]	20.4 ± 4.6	38.2 ± 7.2	0.37
17	ATB-[His^{12}]Svg$_{(12-40)}$	142.5 ± 22.3	3.1 ± 0.2	32.26
18	[Tyr^{11}, His^{12}]Svg$_{(11-40)}$	220.9 ± 89.1	4.9 ± 1.8	31.64
19	[Tyr^{11}]Svg$_{(11-40)}$	977.2 ± 551.0	20.8 ± 1.6	32.97
20	[Tyr^{12}]Svg$_{(12-40)}$	995.5 ± 336.8	428.5 ± 118.9	1.63

[a], astressin = cyclo(30-33)[Nle^{21,38}, Glu^{30}, Lys^{33}]h/rCRF$_{(12-41)}$. [b], ATB = 4-(1-azi-2,2,2-trifluoroethyl)benzoyl. [c], [Ala^{32}]astressin = cyclo(30-33)[Nle^{21,38}, Glu^{30}, Ala^{32}, Lys^{33}]h/rCRF$_{(12-41)}$. [d], [Tyr^{32}]astressin = cyclo(30-33)[Nle^{21,38}, Glu^{30}, Tyr^{32}, Lys^{33}]h/rCRF$_{(12-41)}$.

Selectivity was calculated according to the equation: Selectivity = $K_{d(rCRFR1)}$ (agonist/antagonist)/$K_{d(rCRFR1)}$ (astressin) x $K_{d(mCRFR2\beta)}$ (astressin)/$K_{d(mCRFR2\beta)}$ (agonist/antagonist).

TABLE 4

Potency of 1 μM CRF antagonist to stimulate cAMP production in transfected HEK 293 cells (rel. IA, relative intrinsic activity). Efficacy of 1 μM (rCRFR1) or 10 nM (mCRFR2β) CRF antagonist to inhibit cAMP production stimulated by oCRF (rCRFR1) or Svg (mCRFR2β) (1 nM) in transfected HEK 293 cells (rel. pot., relative potency).

No.	peptides	HEK-rCRFR1 cells		HEK-mCRFR2β cells	
		rel. IA	rel. pot.	rel. IA	rel. pot.
1	oCRF	1.00	1.00	-	-
2	Svg	-	-	1.00	1.00
3	astressin	0.10 ± 0.02	0.11 ± 0.03	0.004 ± 0.001	0.57 ± 0.04
11	[DPhe^{11}, His^{12}]Svg$_{(11-40)}$	0.04 ± 0.01	0.73 ± 0.07	0.004 ± 0.001	0.42 ± 0.02
12	[DPhe^{11}]Svg$_{(11-40)}$	0.07 ± 0.01	0.75 ± 0.13	0.005 ± 0.001	0.71 ± 0.04
13	[DLeu^{11}]Svg$_{(11-40)}$	0.22 ± 0.04	0.92 ± 0.16	0.008 ± 0.002	0.75 ± 0.06
14	Svg$_{(11-40)}$	0.14 ± 0.02	0.76 ± 0.16	0.005 ± 0.001	0.76 ± 0.02
15	ATB-[Ala^{32}]astressin^{b,c}	0.10 ± 0.01	0.11 ± 0.05	0.010 ± 0.001	0.30 ± 0.05
16	ATB-[Tyr^{32}]astressin^{d}	0.08 ± 0.01	0.49 ± 0.16	0.008 ± 0.003	0.68 ± 0.17
17	ATB-[His^{12}]Svg$_{(12-40)}$	0.15 ± 0.01	0.54 ± 0.06	0.007 ± 0.002	0.48 ± 0.04
18	[Tyr^{11}, His^{12}]Svg$_{(11-40)}$	0.03 ± 0.01	0.79 ± 0.07	0.008 ± 0.002	0.37 ± 0.05
19	[Tyr^{11}]Svg$_{(11-40)}$	0.09 ± 0.01	0.82 ± 0.06	0.007 ± 0.002	0.67 ± 0.04
20	[Tyr^{12}]Svg$_{(12-40)}$	0.06 ± 0.03	0.90 ± 0.02	0.006 ± 0.002	0.80 ± 0.04
	basal	0.01 ± 0.003		0.004 ± 0.001	

a, astressin = cyclo(30-33)[Nle^{21,38}, Glu^{30}, Lys^{33}]h/rCRF$_{(12-41)}$. *b*, ATB = 4-(1-azi-2,2,2-trifluoroethyl)benzoyl. *c*, [Ala^{32}]astressin = cyclo(30-33)[Nle^{21,38}, Glu^{30}, Ala^{32}, Lys^{33}]h/rCRF$_{(12-41)}$. *d*, [Tyr^{32}]astressin = cyclo(30-33)[Nle^{21,38}, Glu^{30}, Tyr^{32}, Lys^{33}]h/rCRF$_{(12-41)}$.

TABLE 5
Physiocochemical data of the CRF-analogs.

No.	peptides	RPHPLC[a]	R_t [min][b]	MS (average) calcd/found[c]
1	oCRF	99	25.9	4671.4/4669.2
2	Svg	99	25.4	4600.4/4599.4
3	astressin	99	24.8	3564.2/3563.3
4	α-helical $CRF_{(9-41)}$	97	26.1	3827.4/3826.0
5	[DPhe[12], Nle[21,38]]α-hCRF$_{(12-41)}$	92	24.4	3540.2/3539.1
6	[DPhe[12]]oCRF$_{(12-41)}$	89	23.0	3490.1/3487.6
7	[DPhe[11]]rUcn$_{(11-40)}$	99	24.5	3641.2/3640.8
8	[DPhe[11], Glu[12]]rUcn$_{(11-40)}$	99	25.3	3633.2/3629.9
9	[DLeu[11], Glu[12]]rUcn$_{(11-40)}$	94	25.1	3599.1/3596.4
10	cyclo(29-32)[DPhe[11], Glu[29], Lys[32]] rUcn$_{(11-40)}$	99	24.7	3596.1/3593.2
11	[DPhe[11], His[12]]Svg$_{(11-40)}$	97	21.6	3652.3/3650.3
12	[DPhe[11]]Svg$_{(11-40)}$	96	22.1	3644.3/3642.4
13	[DLeu[11]]Svg$_{(11-40)}$	98	22.0	3610.3/3608.5
14	Svg$_{(11-40)}$	99	22.0	3610.3/3608.5

[a], Percent purity determined by RPHPLC using solvent system: A = 0.1% TFA in water, B = 80% MeCN in 0.1% TFA in water. [b], The retention time (R_t) of the compounds was determined by RPHPLC. Samples were eluted with 5% B for 5 min and then with a linear gradient of 5-95% B in 30 min. [c], The observed *m/z* of the average compared with the calculated $[M+H]^+$ average mass.

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Claims

1. An antagonist of the ligand of the Corticotropin-Releasing Factor Receptor, type 2 (CRFR2) lacking the 8 to 10 N-terminal amino acids of native sauvagine.

2. The antagonist of claim 1 lacking the 10 N-terminal amino acids of native sauvagine.

3. The antagonist of claim 2 comprising the amino acid sequence Xaa_1-Xaa_2-Leu-Leu-Arg-Lys-Met-Ile-Glu-Ile-Glu-Lys-Gln-Glu-Lys-Glu-Lys-Gln-Gln-Ala-Ala-Asn-Asn-Arg-Leu-Leu-Leu-Asp-Thr-Ile-NH_2, wherein Xaa_1 is a neutral amino acid, and Xaa_2 is a charged amino acid.

4. The antagonist of claim 3, wherein Xaa_1 is a hydrophobic amino acid, and Xaa_2 is Glu or His.

5. The antagonist of claim 4, wherein Xaa_1 is Leu.

6. The antagonist of claim 3, wherein Xaa_1 is a polar amino acid, and Xaa_2 is Glu or His.

7. The antagonist of claim 6, wherein Xaa_1 is Tyr.

8. The antagonist of any one of claims 3, 4 or 6, wherein Xaa_1 is in the D-configuration

9. The antagonist of claim 8, wherein Xaa_1 is D-Leu.

10. The antagonist of claim 8, wherein Xaa_1 is D-Tyr.

11. The antagonist of any one of claim 5, 7, 9 or 10, wherein Xaa_2 is Glu.

12. The antagonist of claim 8, wherein Xaa_1 is D-Phe.

13. The antagonist of any one of claims 7, 10 or 12, wherein Xaa_2 is His.

14. An antagonist of the ligand of the Corticotropin-Releasing Factor Receptor, type 2 (CRFR2) lacking the 11 N-terminal amino acid of native sauvagine, wherein the N-terminal amino acid of said antagonist is a charged amino acid.

15. The antagonist of claim 14, wherein said charged amino acid is positively charged.

16. The antagonist of claim 15, wherein said charged amino acid is His.

17. The antagonist of any one of claims 14 to 16 which comprises a phenyldiazirine group coupled to the N-terminal amino acid of said antagonist.

18. The antagonist of claim 17, wherein said phenyldiazirine group is a 4-(1-azi-2,2,2-trifluoroethyl)benzoyl (ATB)-group.

19. A polynucleotide encoding the antagonist of any one of claims 1 to 7, 11, 13 or 14 to 16.

20. A vector comprising the polynucleotide of claim 19.

21. The vector of claim 20, wherein said polynucleotide is operatively linked to an expression control sequence.

22. A host comprising the polynucleotide of claim 19 or vector of claim 20 or 21.

23. A method for producing the antagonist of any one of claims 1 to 7, 11, 13, or 14 to 16, said method comprising culturing the host of claim 22 under conditions that cause the synthesis of said antagonist, and recovering said antagonist from the culture.

24. An antagonist obtainable by the method of claim 23.

25. An antibody directed against the antagonist of any one of claims 1 to 18, or 24.

26. An anti-idiotypic antibody directed against the antibody of claim 25.

27. A pharmaceutical composition comprising the antagonist of any one of claims 1 to 18, or 24, the polynucleotide of claim 19, the vector of claim 20 or 21, the antibody of claim 25 and/or the anti-idiotypic antibody of claim 26 and optionally a pharmaceutically acceptable carrier and/or diluent.

28. A diagnostic composition comprising the antagonist of any one of claims 1 to 18, or 24, the polynucleotide of claim 15, the vector of claim 20 or 21, the antibody of claim 45 and/or the anti-idiotypic antibody of claim 26.

29. A kit comprising
    (a) an antagonist of any one of claims 1 to 18, or 24;
    (b) the polynucleotide of claim 19;
    (c) the vector of claim 20 or 21;
    (d) the antibody of claim 25; and/or
    (e) the anti-idiotypic antibody of claim 26.

30. Use of the antagonist of any one of claims 1 to 18, or 24, the polynucleotide of claim 19, the vector of claim 20 or 21, the antibody of claim 25 and/or the anti-idiotypic antibody of claim 26 for the preparation of a pharmaceutical composition for preventing and/or treating a Corticotropin-Releasing Factor Receptor, type 2 (CRFR2)-associated disease.

31. The antagonist of any one of claims 1 to 18, or 24, or the use of claim 30, wherein said CRFR2 is CRFR2α or CRFR2β.

32. The use of claim 30 or 31 for preventing and/or treating affective disorders,

gastric intestinal diseases, cardiopathic diseases, psychiatric diseases, preferably eating disorders, anxiety disorders or anorexia nervosa, and/or Alzheimer's disease.

33. Use of the antagonist of any one of claims 1 to 18, or 24, the polynucleotide of claim 19, the vector of claim 20 or 21, the antibody of claim 25 and/or the anti-idiotypic antibody of claim 26 for the investigation of CRF receptor type-specific functions.

Abstract

The present invention relates to an antagonist of the ligand of the Corticotropin-Releasing Factor Receptor, type 2 (CRFR2) lacking the 8 to 10 N-terminal amino acids of native sauvagine. The present invention also relates to an antagonist of the ligand of the Corticotropin-Releasing Factor Receptor, type 2 (CRFR2) lacking the 11 N-terminal amino acids of native sauvagine, wherein the N-terminal amino acid of said antagonist is a charged amino acid. Furthermore, the present invention relates to a polynucleotide encoding the antagonist of the present invention, a vector comprising the polynucleotide of the present invention, and a host comprising the polynucleotide or the vector of the present invention. Also described are a method for producing the antagonist of the present invention, antibodies directed against the antagonist of the present invention, as well as anti-idiotypic antibodies directed against the antibody of the present invention. The present invention also relates to pharmaceutical and diagnostic compositions comprising the antagonist, the polynucleotide, the vector, the antibody, and/or the anti-idiotypic antibody of the present invention. Furthermore, the present invention relates to a kit comprising one or more of the above mentioned compounds of the present invention and to the use of one or more of these compounds for the preparation of a pharmaceutical composition for preventing and/or treating a Corticotropin-Releasing Factor Receptor, type 2 (CRFR2)-associated disease.

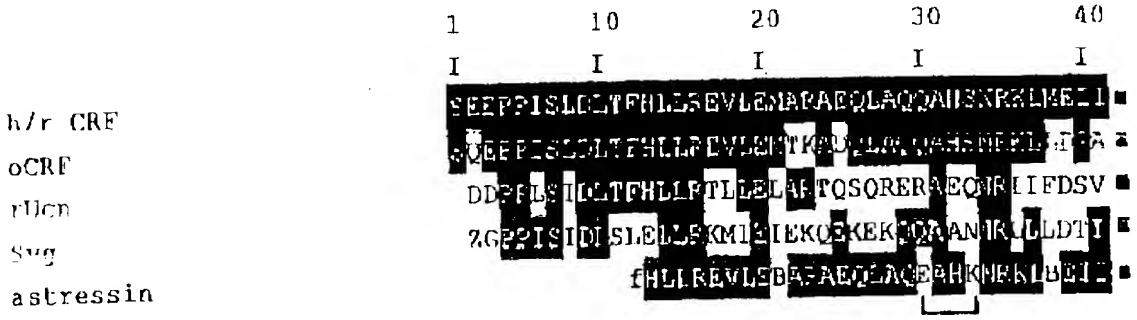


Fig. 1

Z = pyroglutamic acid

f = D-phenylalanine

B = norleucine

└──┘ = lactam bridged

■ = NH_2

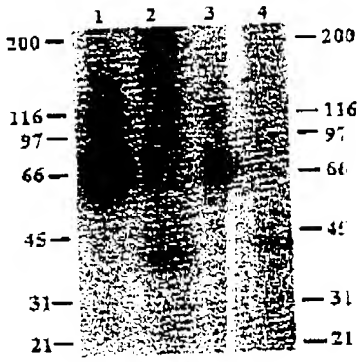
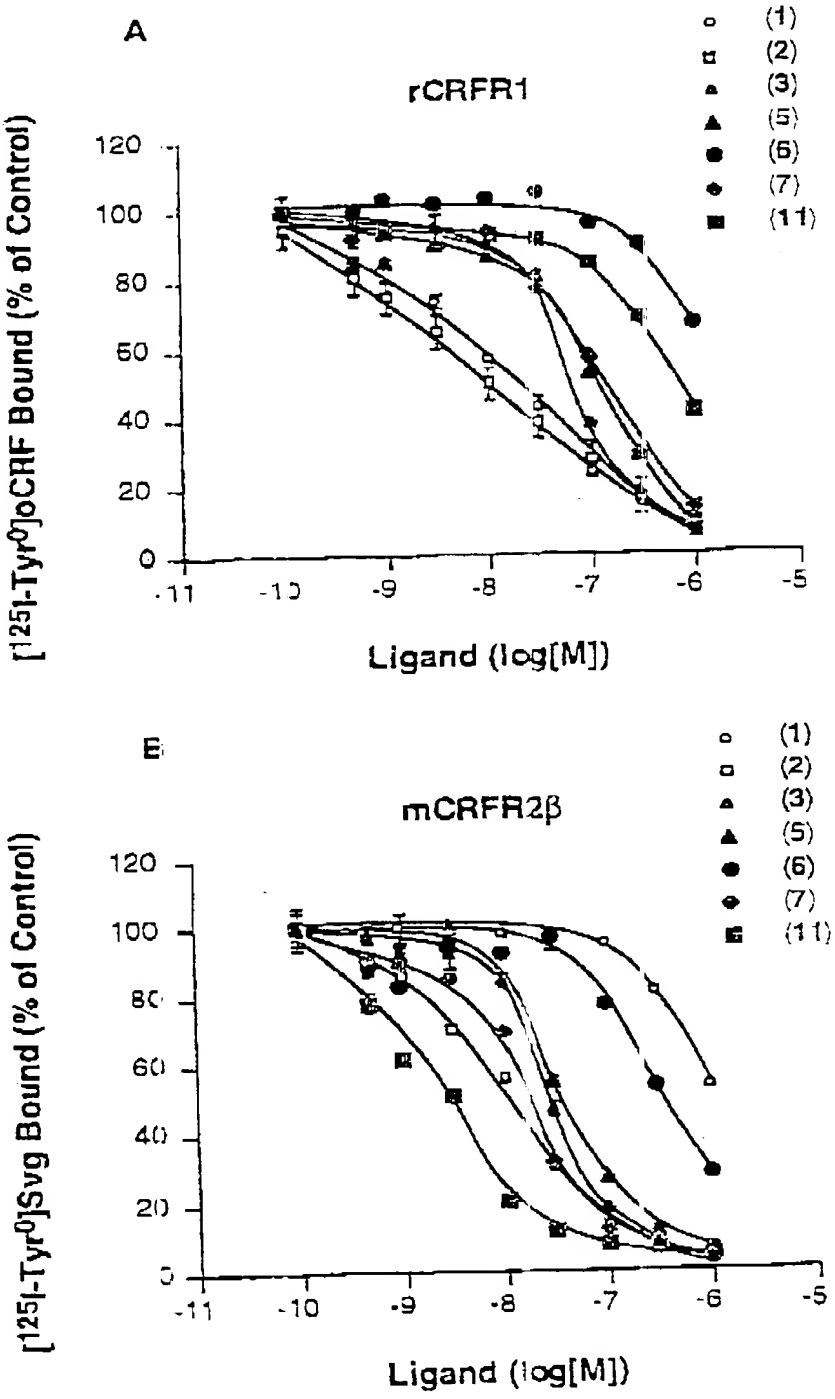


Fig. 2


A
rCRFR1
(1)
(2)
(3)
(5)
(6)
(7)
(11)
[125I-Tyr0]oCRF Bound (% of Control)
120
100
80
60
40
20
0
-11
-10
-9
-8
-7
-6
-5
Ligand (log[M])
B
mCRFR2β
(1)
(2)
(3)
(5)
(6)
(7)
(11)
[125I-Tyr0]Svg Bound (% of Control)
120
100
80
60
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20
0
-11
-10
-9
-8
-7
-6
-5
Ligand (log[M])

Fig. 3

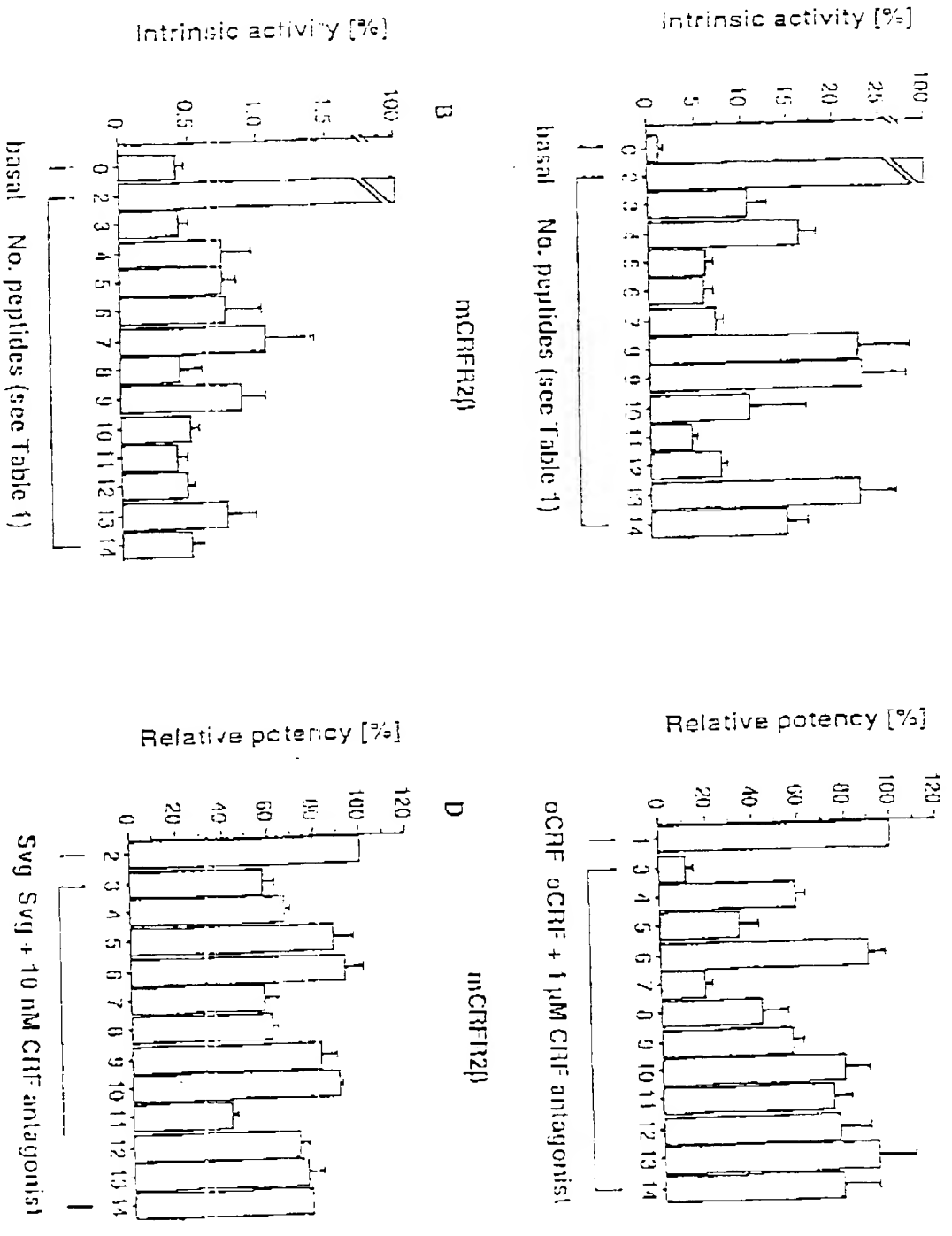
A
rCRFR1
Intrinsic activity [%]
basal
No. peptides (see Table 1)
B
mCRFR2β
Intrinsic activity [%]
basal
No. peptides (see Table 1)
C
rCRFR1
Relative potency [%]
oCRF
oCRF + 1 μM CRF antagonist
D
mCRFR2β
Relative potency [%]
Svg
Svg + 10 nM CRF antagonist

Fig. 4